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4703

Variation of Response to Infection With Treponema Pallidum Between an Albino and a Brown Breed of Rabbit.

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This experiment was undertaken for the purpose of comparing the course of syphilitic infection in 2 different breeds of rabbit, one a pure albino and the other a brown variety, inoculated under identical conditions with a virulent strain of *Treponema pallidum.* The animals were normal young males. The body weight of the albinos varied from 1430 to 1860 gm., that of the brown rabbits from 1340 to 2130 gm. During the experiment they were housed under uniform conditions in a light and well ventilated room, and fed on the same diet.

Experimental. The strain of *Treponema pallidum* used was that originally isolated by Nichols[1] from the spinal fluid of a patient with syphilitic neurorecurrence. This strain, now 17 years old, has had many passages through rabbits and has attained a high degree of virulence for this animal. The strain was brought to China in September, 1927. The immediate source of virus for this experiment was one of the third group of rabbits through which the strain had passed in this country. This animal was a brown breed and showed clinical evidence of orchitis 28 days after inoculation. When the orchitis was progressing, the right testis was excised under ether anesthesia, finely minced with scissors, and ground up in a sterile mortar with sterile physiologic sodium chloride solution. Of the more fluid portion of this emulsion, 0.3 cc. was inoculated into the right testis of each of 8 albino and 9 brown rabbits. The inoculations were all made on February 24, 1928. The emulsion contained from 2 to 3 actively motile organisms to the microscopic field. One animal in each group of rabbits died of pulmonary disease soon after inoculation. These are not included in the results of the experiment.

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[1] Nichols, H. J., *J. Exp. Med.*, 1914, xix, 362.

Results. The data on the natural course and character of the infection among the individual animals in the 2 groups (Group A—Albino, Group B—Brown) have been tabulated in the accompanying table.

TABLE I.

Comparison of Primary and Metastatic Lesions in Albino and Brown Rabbits Inoculated Intratesticularly with Treponema Pallidum.

Rabbit	Primary Orchitis		Metastatic Lesions					Period of Observation
Group A Albino	Time of Appearance	Duration days	Testis		Bone	Skin	Eye	
			Orchitis	Duration				
				days				days
1	+(11)	77	+(63)	74	0	0	+(230, 298, 324,* 338*)	338
2	+(11)	84	+(60)	10	0	0	+(95)	338
3	+(11)	168	+(109)	21	+(46)	0	+(165)	179
4	+(11)	112	+(42)	67	0	0	+(123,300)	338
5	+(14)	95	+(53)	56	0	0	0	258
6	+(11)	84	+(63)	59	+(56)	0	+(130)	338
7	+(14)	158	0		0	0	+(74,130)	186
Average	(11.5)	111.1	(55.7)	47.8	+28%	0	+85.7%	
Gr. B Brown								
8	+(7)	88	+(53)	42	+(46)	0	+(223)	338
9	+(11)	119	+(46)	49	0	0	0	172
10	+(14)	32	+(26)	12	0	0	0	338
11	+(11)	56	+(60)	10	0	0	0	338
12	+(14)	81	+(95)	14	0	0	0	195
13	+(14)	81	0		0	0	0	167
14	+(18)	26	+(39)	14	0	0	0	338
15	+(14)	74	+(42)	60	+(56)	+(42)	0	338
Average	(12.8)	69.6	(45)	28.5	+25%	+12.5%	+12.5%	

* Recurrent keratitis.

The figures in parenthesis indicate the number of days after inoculation.

All of the animals developed a typical syphilitic orchitis of the inoculated testis from 11 to 18 days after inoculation. There was no significant difference between the 2 groups in the length of the period of incubation; however, the primary reaction of the albinos was characterized by a much greater enlargement and induration of the testis than that of the brown rabbits. All but one of the former developed scrotal chancres from 1 to 3 cm. in diameter, which healed slowly, while only 2 of the brown animals had scrotal ulcers comparable to those of the albinos. The average duration of the primary lesions was significantly longer in the albino than in the brown group.

The incidence and character of the metastatic testicular lesions in both groups were generally uniform, although those of the albino rabbits were of somewhat longer duration. There were 2 cases of periosteal and bone lesions in each group, and one metastatic skin lesion among the brown animals.

It was in the number and character of the metastatic eye lesions that the two breeds presented the most remarkable variations in their respective reactions to the infection. Six of the 7 albino rabbits manifested syphilitic lesions of the eyes.[2] With one exception (Rabbit 2) all of these were well defined cases of interstitial keratitis. In the case of Rabbit 2 there was pronounced ciliary congestion without clinically detectable involvement of the cornea or iris. In Group A there were several instances of transient mild degrees of pericorneal vascular injection which have not been included in the accompanying table. Rabbit 9 was the only animal of brown breed that developed keratitis. Rabbit 15 of the same group had a slight transient congestion of the right eye over the superior rectus muscle at its corneal margin, which was of doubtful significance. Otherwise there was no other instance of clinically detectable ocular lesions in Group B.

During the period of the most active clinical lesions the albino rabbits were in poor general condition. They were less active than before the onset of the disease and their hair lost its usual sleekness, turning a dirty yellow color in irregular patches on the abdomen, flanks and legs. The brown animals remained vigorous and well groomed.

The animals were under observation for a period of 338 days after inoculation, with the exception of 3 rabbits of each group that died before the expiration of this time at intervals of from 167 to 258 days after inoculation. The deaths of 5 of these occurred in the month of August following severe diarrhoea. No gross lesions of significance were found at necropsy. Since the only case of keratitis in Group B occurred as late as 223 days after inoculation it is possible that had they survived longer 3 of the brown animals might have developed similar lesions. It is to be noted, however, that 4 animals of this group, in addition to Rabbit 8 of the brown breed, which reacted with a keratitis, survived for the full period of the experiment without showing lesions of the eyes.

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[2] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1921, xxxiv, 167.

Recurrence of generalized metastatic lesions occurred only in Rabbit 1 in the form of keratitis of both eyes.

Conclusion: The evidence as presented above indicates that under the conditions of the experiment the albino rabbits offered less resistance to infection with *Treponema pallidum* than did the brown breed of rabbit. This difference was manifested particularly in the magnitude and duration of the primary orchitis and in the incidence of metastatic keratitis. The albino group with more pronounced primary testicular reactions developed a high incidence of eye lesions. Six of the 7 animals, or 85.7%, developed metastatic lesions of the eye, while in the brown group whose primary response to infection was less pronounced, one out of 8 animals, or 12.5%, manifested ocular lesions.

While other factors of which we are not cognizant may have entered into the conditions influencing the course of the disease in these rabbits, the one known variable was that of breed. Whether the variations in the disease phenomena observed between the pigmented and non-pigmented breeds would be found constant, or relatively so, under similar conditions is a doubtful question. It is worthy of note, however, that in this experiment clinical variations occurred during the natural course of the disease where no attempt was made to modify the defensive mechanism of the hosts following unilateral intratesticular inoculation with equal amounts of the same virus at the same time. These divergent reactions must be attributed to some inherent constitutional factor of the host and not to strain specificity of the infecting organism.

4704

Distribution of Protein Fractions in the Serum of Kala-azar Patients.

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By applying Ray's "hemolytic" test,[1] Sia[2] found that the blood of Kala-azar patients always hemolizes to a cloudy solution which on standing deposits a flocculent precipitate, whereas in normal subjects and in other patients studied, including severe anemia, it hemolizes to a clear solution which remains clear. Later Sia and Wu[3] showed that the turbidity is due to serum globulin and that Ray's "hemolytic" test is really a globulin precipitation test for Kala-azar. They also found that there is, in the blood of Kala-azar patients, an absolute decrease of serum albumin and an absolute increase of serum globulin. Since the test is performed by adding 20 cmm. of blood to 0.6 cc. of distilled water,[4] and since euglobulin is precipitated simply by dilution with water, it is desirable to know whether the turbidity and precipitate are determined by the amount of euglobulin in the serum. We have, therefore, made quantitative determinations of different fractions of serum protein in normal subjects and in Kala-

TABLE I.
The distribution of the protein fractions in normal human serum.
Gm. in 100 cc. of serum.

Subject No.	Albumin (a)	Euglobulin (b)	Pseudoglobulin		Total globulin (e) (b+c+d)	Euglobulin	Total protein		Globulin
			I (c)	II (d)		Total globulin (b:e)	Determined	Calculated (a+e)	Albumin (e:a)
1	4.310	0.294	1.042	0.698	2.034	0.144	6.344	6.344	0.472
2	4.309	0.305	1.224	0.768	2.297	0.132	6.606	6.606	0.533
3	4.391	0.300	1.169	0.667	2.136	0.140	6.525	6.527	0.486
4	4.137	0.323	0.969	0.646	1.938	0.166	6.075	6.075	0.468
5	3.950	0.323	1.007	0.797	2.127	0.152	6.077	6.077	0.538
6	4.194	0.284	1.158	0.676	2.118	0.134	6.312	6.312	0.505
7	4.010	0.234	0.919	0.590	1.743	0.134	5.754	5.753	0.435
8	4.665	0.333	1.087	0.514	1.934	0.172	6.598	6.599	0.415
9	4.009	0.323	0.969	0.736	2.028	0.159	6.035	6.037	0.506
10	4.086	0.326	1.161	0.646	2.133	0.153	6.218	6.219	0.522
Average	4.206	0.305	1.071	0.674	2.049	0.149	6.254	6.255	0.487

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[1] Ray, C. B., *Indian Med. Gaz.*, 1921, lvi, 9.

[2] Sia, R. H. P., *China Med. J.*, 1921, xxxv, 397.

[3] Sia, R. H. P., and Wu, H., *China Med. J.*, 1921, xxxv, 527.

[4] Sia, R. H. P., *China Med. J.*, 1924, xxxviii, 35.

azar patients. In all cases of Kala-azar the globulin test by Sia's method was positive and stained smears made from splenic pulp showed Leishman-Donovan bodies.

The serum proteins were fractionated with different concentrations of sodium sulphate according to the technic of Howe.[5] Tables I and II give the results of determinations on normal subjects and

TABLE II.

The distribution of the protein fractions in the serum of Kala-azar.
Gm. in 100 cc. of Serum.

Hospital No.	Albumin (a)	Euglobulin (b)	Pseudoglobulin		Total globulin (e) (b+c +d)	Euglobulin	Total protein		Globulin	"Globulin test"
			I (c)	II (d)		Total globulin (b:e)	Determined	Calculated (a+e)	Albumin (e:a)	
22348	3.049	2.353	1.324	0.627	4.304	0.547	7.353	7.353	1.412	++++
5166	2.451	1.050	1.697	0.754	3.501	0.299	5.952	5.952	1.428	++
24659	1.191	2.269	2.372	0.748	5.389	0.421	6.579	6.580	4.524	++++
24753	1.250	2.982	2.704	0.417	6.103	0.488	7.353	7.353	4.882	++++
25242	2.745	0.960	1.468	0.359	2.787	0.344	5.532	5.532	1.015	+++
24736	2.712	1.510	2.166	0.551	4.227	0.357	6.939	6.939	1.558	++++
25573	1.838	2.153	1.806	0.563	4.522	0.476	6.358	6.360	2.460	++++
25547	2.370	2.088	1.500	0.481	4.069	0.513	6.438	6.439	1.717	++++
25638	3.231	3.583	1.553	0.505	5.641	0.635	8.873	8.872	1.745	++++
26043	1.639	3.969	1.910	0.518	6.397	0.620	8.036	8.036	3.903	++++

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[5] Howe, P. E., *J. Biol. Chem.*, 1921, xlix, 109.

on patients with Kala-azar respectively. By comparing the figures in these 2 series it will be seen that euglobulin in Kala-azar serum is increased 3 to 13 times and amounts to 30 to 63% of the total serum globulin; the pseudo-globulin I is nearly twice, in some cases more than twice, as much as in normal serum. There is a considerable increase of total serum globulin and an absolute decrease of serum albumin, so that the globulin:albumin ratio, which is normally about 0.49, is greatly raised, being in some cases over 4. Findings somewhat similar to these were made by Lloyd and Paul[6] who found, in 8 cases of Kala-azar, euglobulin to be 1.5 to 2.5 gm. per 100 cc. of serum, but apparently without change in pseudo-globulin. In view of these results such a precipitation in the globulin test may be considered as due to increase of euglobulin, or some special euglobulin, in the Kala-azar serum.

4705

Cytological Changes in Thyroid Apparatus and Spinal Ganglia of Rats Treated With Thallium.

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Buschke[1] and others believe that thallium acetate acts chiefly on the thyroid gland and the nervous system. Balbi,[2] however, is of the opinion that it does not have such action. Since the Golgi apparatus and the mitochondria are the most vital cellular elements known so far, it is suggested that a study of the action of thallium acetate on these elements in the 2 tissues in question should yield more definite information than ordinary histological examination of the tissue as a whole. The following observations have been made:

Eighteen albino rats of about the same age (3.25 to 3.5 months) and sex (male) were injected with a single injection of 8 mg. of thallium acetate per kilo of body weight and killed at weekly intervals thereafter for the study of the Golgi apparatus, mitochondria and other cellular elements in various organs and tissues. For each experiment, 3 animals were employed.

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[6] Lloyd, R. B., and Paul, S. N., *Indian J. Med. Res.*, 1928, xvi, 529.

[1] Buschke, A., Langer, E., and Peiser, B., *Dermat. Wchnschr.*, 1926, lxxxiii, 971.

[2] Balbi, E., *Gior. ital. di dermat. e. sifil.*, 1928, lxix, 28. (Abstract, *Brit. J. Dermat. and Syph.*, 1929, xli, 78.)

Normally, the spinal ganglion cells contain a network-like Golgi apparatus and long and short, rod-like mitochondria interspersed with Nissl bodies concentrically around the nuclei. In the thyroid cells the network-like Golgi apparatus lies on the luminal side of the nucleus and the mitochondria run parallel along the long axis of the cells in the basal portions. The colloid substance in the thyroid vesicles exhibits a basophilic staining reaction.

The cellular changes after the administration of thallium acetate are summarized as follows: *One week* after the injection the Golgi apparatus in some of the thyroid cells is changed from network into droplets, the mitochondria from filaments into segmented granules, and the staining property of the colloid substance from basophilic to an acidophilic reaction. *Two weeks* after the injection the droplets and granules of the Golgi apparatus and mitochondria became much finer in all the cells and were found dispersed in the distal portions of the cells. The colloid substance in the thyroid vesicles was more acidophilic in staining reaction. *Three weeks* after the injection the changes in both the Golgi apparatus and the mitochondria were still more marked and these elements were also reduced in number. The thyroid cells became definitely flattened with very poorly stained nuclei. Many vacuoles were found in the acidophilic colloid substance. *Four weeks* after the injection both the Golgi apparatus and the mitochondria tended to exhibit a normal appearance, inasmuch as these elements could be clearly stained in the form of short rods and granules. The colloid secretion, however, still remained acidophilic. *Six weeks* after the injection complete recovery of all the cellular structures was observed.

The changes in the Golgi apparatus and the mitochondria occurring in the spinal ganglion cells were more or less similar to those observed in the thyroid cells. Marked chromatolysis was noticed 3 weeks after the injection and complete disappearance of the Nissl substance in many of the cells 4 weeks after the injection. Recovery of all the cellular structures commenced to appear 6 weeks after the injection.

Summary. From the above observations, it is clearly indicated that the administration of 8 mg. of thallium acetate per kilo of body weight to albino rats produced definite changes in the Golgi apparatus and mitochondria of the thyroid and spinal ganglion cells and in the Nissl substance of the spinal ganglion cells, and altered the staining property of the colloid secretion of the thyroid vesicles from basophilic to acidophilic reaction over a period of 5 weeks after the injection. These findings, however, gradually began to disappear and complete recovery took place 6 weeks after the injection.

4706

Effect of Thallium Acetate on the Basal Metabolism of Rats.

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Leigheb[1] and others have observed that the administration of thallium acetate to rats and guinea-pigs produces extensive degenerative changes in the thyroid gland. In view of these observations, the following experiments were undertaken to study the basal metabolism of animals treated with the drug.

Four white rats of the same breed and of approximately the same age were injected subcutaneously with thallium acetate in aqueous solution. Three of the animals received 12 mg. of the thallium salt per kilo of body weight and one, 8 mg. per kilo. Before the administration of the thallium acetate, 4 observations were made on the basal metabolism of each animal at intervals of 3 or 4 days. After the administration of the drug, the basal metabolism was studied at similar intervals, until the basal metabolic rate returned to normal. The technic used for determining the basal metabolism was that described by Wu and Chen.[2] For each experiment there were from 6 to 8 determinations. The average of only those determinations (usually 3) taken at the time the animal was quiet was used as the final result of the experiment.

All of the rats showed diminution of their basal metabolic rates, in parallel with which, defluvium of hair on the back and less briskness of the animals were noticed. In the 3 rats injected with 12 mg. of thallium acetate per kilo of body weight, the basal rates began to diminish from the 8th to the 15th day after the administration of the thallium salt. One of the animals died at the end of the experiment on the 21st day after the injection of the drug, at which time the basal metabolic rate was at its lowest. Post-mortem examination revealed no gross abnormality in the organs, but histological examination showed flattening of the cells in the thyroid gland. In the other 2 rats, the decrease in rate persisted for 55 and 66 days and returned to normal on the 70th and 74th day of the experiment respectively. In one rat the rate on the 25th day was 32% less than its normal, at which time there was marked falling of hair. The observations on this animal are illustrated by curve B on the chart. In the rat injected with 8 mg. of thallium acetate per kilo of body weight, the decrease of the basal metabolic rate commenced on the 6th day after injection and lasted for 33 days. Return to normal was reached on the 39th day. The lowest rate, which was 25% less than the normal, occurred on the 19th day and was accompanied by extensive defluvium of hair. Curve A on the chart shows the variations of rate observed in this animal.

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[1] Leigheb, V., *Gior. ital. di dermat. e. sifil.*, 1928, lxix, 960. (Abstract, *Brit. J. Dermat. and Syph.*, 1929, xlvi, 129, and *Arch. Dermat. and Syph.*, 1929, xix, 295.)

[2] Wu, H., and Chen, T. T., *Chin. J. Physiol.*, 1929, iii, 307.

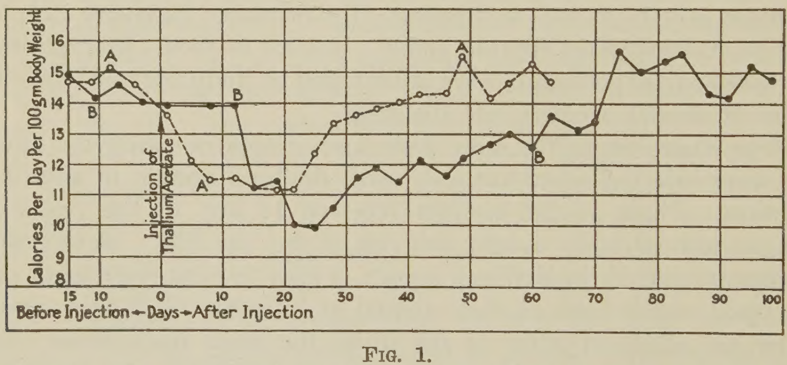

FIG. 1.
Variation of metabolic rate in rats A and B following the administration of 8 mg. and 12 mg. of thallium acetate per kilogram of body weight respectively.

Summary. Thallium acetate when injected subcutaneously into rats in doses of 12 and 8 mg. per kilo of body weight caused definite but transitory diminution of their basal metabolic rates between the second and eleventh weeks after the administration of the drug. The diminution was more marked and more persistent following the use of the larger dose. During the time of the depression of the basal metabolic rate, the animals became inactive and there was also falling of hair, particularly when the metabolism was at its lowest level. In view of the observations in these experiments and the morphologic cellular changes observed in the Golgi complex and mitochondria of the thyroid gland,[3] we are inclined to attribute the variations of the metabolic rate to direct action of the thallium on the thyroid gland.

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[3] Ma, W. C., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, —?

4707

Action of Ephedrine and Pseudoephedrine Upon the Bronchial Muscle.

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Using our modified method of the isolated lung perfusion described by Sollmann and Von Oettingen,[1] we studied the peripheral action of ephedrine and pseudoephedrine on rabbit bronchial muscle. The arrangement of the perfusion was similar to that of Sollmann and Von Oettingen, but the borders of the lung were trimmed with sharp scissors so that the perfusion fluid was allowed to drain freely. The lung was lightly supported with a funnel covered with moist cotton to prevent drying, drops per minute through the funnel being reckoned as the rate of the bronchial outflow. The drugs took from half to one minute to reach the outflow, and 4 to 5 minutes for the concentration of drug of the outflow to be the same as that of the inflow. After 1 to 2 hours of perfusion with Tyrode solution, when the rate of outflow was 50 to 80 drops per minute, and when it had remained nearly constant for over 10 minutes, the perfusion of the ephedrine-Tyrode solution was started, and allowed to continue for 20 to 30 minutes (pH of ephedrine and Tyrode solution was found to be the same). Bronchial constriction was indicated by a decrease of outflow, and dilation by an increase. The bronchial muscle under this condition was found to be exceedingly sensitive to drugs, comparing with the method of Sollmann and Von Oettingen. After 6 to 7 hours of perfusion the bronchioles still responded to drugs. Injection of 0.5 cc. of pilocarpine 1:1,000,000 or atropine 1:1,000,000 into the connecting tube between lung and perfusion apparatus caused respectively a distinct constriction and dilation of the bronchioles.

The response of the bronchial muscle to ephedrine varied with the dosage. Perfusion with highly diluted concentrations, such as 1:1,000,000 to 1:5,000,000 in 6 experiments produced a definite bronchial dilatation, and the effect of 1:1,000,000 was more distinct than that of the more dilute solution. Comparing this effect with the action of ephedrine on the circulation and other smooth muscles the bronchial dilation is probably a sympathetic action. Perfusion with moderate concentrations of ephedrine, namely 1:10,000 to 1:200,000, in 7 experiments uniformly caused bronchial constriction, and the effect of 1:10,000 was stronger than that of 1:100,000. In 4 experiments, atropine 1:500,000, perfused for 6 to 10 minutes previous to ephedrine, had no influence on the bronchial constricting effect of ephedrine. In this instance the broncho-constricting effect of ephedrine is probably a muscular effect and its sympathetic action is apparently not present. Perfusion with high concentrations, *i. e.,* 1:200 to 1:2,000, in 9 experiments caused either marked constriction (4 cases), or marked dilatation of the bronchioles, depending on the condition of the bronchial muscle and its sensitivity to drugs. The constricting effect was more pronounced than that from moderate concentrations of ephedrine. The marked dilatation may be due to direct muscular depression, as the subsequent injection of barium chloride produced no effect.

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[1] Sollmann, T., and Von Oettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 692.

The effect of pseudoephedrine on the bronchial muscle is similar to that of ephedrine. Concentrations between 1:1,000,000 and 1:100,000, in 5 experiments produced bronchial dilatation in sensitive bronchial preparations, but no effect in insensitive ones. The perfusion with 1:10,000 and with 1:2,000 each in 2 experiments produced a regular distinct bronchial constriction, and the constricting effect of 1:10,000 was apparently stronger than the same concentration of ephedrine. Pseudoephedrine seems to be more musculotropic than ephedrine.

We conclude that ephedrine is a sympathomimetic and musculotropic drug, and this fact confirms further the observation of Pak and Read[2] on the blood pressor action. Tainter[3] and others claimed ephedrine to be a pure musculotropic drug, while on the other hand, Chen[4] and others believed it to be a sympathomimetic amine. These divergent conclusions are possibly due to dissimilar dosage, one may have used large doses in which the sympathetic action was overpowered by strong muscular action, or, on the other hand, small dosage may have been used, which produces a pure sympathetic effect.

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[2] Pak, C., and Read, B. E., *Chinese J. Physiol.,* 1928, ii, 1; 1929, iii, 435.

[3] Tainter, M. L., *J. Pharm. Exp. Therap.,* 1929, xxxvi, 569.

[4] Chen, K. K., *J. Pharm. Exp. Therap.,* 1928, xxxiii, 219.

4708

The Synergistic Action of Benzyl Ephedrine and Adrenaline.

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It has already been stated[1] that benzylephedrine, B-phenyl-B-hydroxy-d-methyl-ethyl-methyl-benzyl-amine, has a local anesthetic action about twice as powerful as procaine, as measured by the wheal method.

The principle of adrenaline-procaine synergism was applied to this new compound. Using 0.05% solutions the addition of 0.0005% adrenaline prolonged the time of wheal anesthesia from 8 to 18 minutes. The further addition of adrenaline up to 0.005% prolonged the time to 140 minutes. The latter is a greater potentiation than is obtained with procaine adrenaline mixtures. Increasing the amount of benzylephedrine prolonged the anesthesia, but greater increase obtained with the higher percentage of adrenaline would

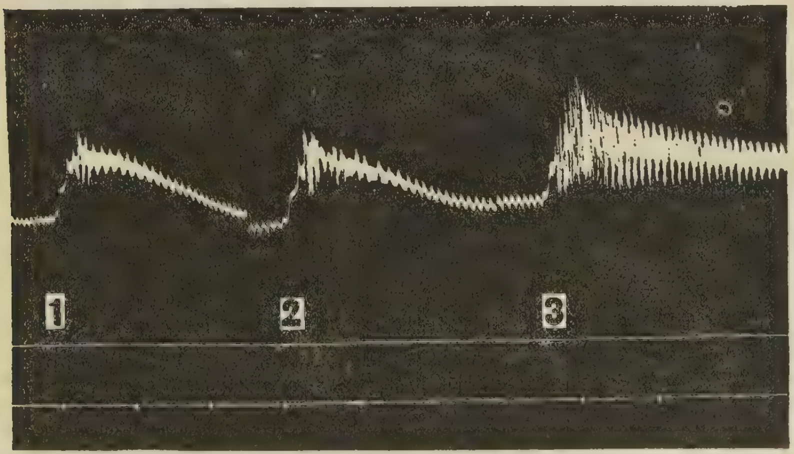

FIG. 1.

A comparison of the actions of benzyl ephedrine and cocaine with adrenaline. Dog ♂. 9.6 kilos. Luminalized 0.15 per kilo.

1. 1 cc. adrenaline, 1 in 100,000, by intravenous injection.
2. Same amount of adrenaline 15 minutes after administering 192 mgm. benzylephedrine (1%) subcutaneously.
3. Same amount of adrenaline 15 minutes after 192 mgm. cocaine (1%) subcutaneously.

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[1] Proceedings of the International Physiological Congress, Boston, August 19, 1929.

indicate that vasoconstriction and subsequently delayed absorption is the more important factor.

The known potentiation of the blood pressure action of adrenaline by cocaine with its excessive vasoconstriction is thought to be the factor in the toxicity of this mixture. Comparative experiments upon luminalized dogs with benzylephedrine show that: 1. Benzylephedrine does not potentiate the blood pressor action of adrenaline as is seen with cocaine (see Fig. 1). 2. The pulse did not show such marked changes as are seen with cocaine-adrenaline reactions. 3. After benzyl-ephedrine-adrenaline produced a slower respiration, which effect was greater than that after cocaine.

Missouri Section.

St. Louis University School of Medicine, December 11, 1929.

4709

Effect of Follicular Extract on Eggs of Fundulus.

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Fundulus eggs covered for 10 minutes with sea water and 5 cc. of follicular extract, F1, 0.13; F2, 0.0026; F3, 0.00086; F4, 0.00043 units per cc., were removed from the solution and fertilized. In each of the first 3 concentrations 85 eggs were used both in the experimental lots and in the controls with the following results:

	No. Fertilized Experimental Lot	No. Fertilized Controls
F1	40	74
F2	41	79
F3	48	71

Of the 80 eggs exposed to F4 follicular extract 57 were fertilized, while the controls yielded 72 fertilized. Of a total of 335 eggs treated with the follicular extract 180 or 53% were fertilized; of 335 controls, 296 or 88%.

Fertilized eggs were placed for 10 minutes in sea water containing 5 cc. F1 follicular extract and then removed to different salt solutions and the time required for the eggs to sink was noted, with the following results:

No. Eggs	Solution	Experimental Lot	Controls
10	2.5 NaCl	7-9 hrs.	3-4½ hrs.
10	2.5 KCl	2-2½ hrs.	10-25 min.
10	1.5 CaCl	1-2 hrs.	25-45 min.
10	24 vols. 2.4 KCl and 1 vol. 1.5 CaCl	50-70 hrs.	45-54 hrs.
10	2/3 sea water	20-25 hrs.	25-35 min

On the second day after fertilization 30 eggs were injected for 6 consecutive days with 0.01 cc. F3 follicular extract and the following changes in the embryo were observed: (a) abnormally dark pigment cells, (b) anastomosis of these cells and streaming of the pigment granules, (c) defective lens in one or both eyes, (d) diminutive forebrain. Practically the same abnormalities appeared in the embryos exposed to 5 cc. of F3 follicular extract placed in the sea water.

Thirty beating hearts stopped within an hour by 0.5 KCl were placed in sea water and 10 cc. F1 follicular extract; within 5 hours they recovered and the controls likewise began to beat in the same length of time. Thirty beating hearts placed in sea water and 10 cc. F1 follicular extract were then exposed to the KCl and within 55 minutes they had all stopped beating, while the controls ceased to beat within 58 minutes.

Conclusions: 1. Under the condition of this experiment dilute solutions of our follicular extract modify profoundly the permeability of the Fundulus egg, apparently in some cases preventing the possibilities of fertilization. 2. Changes in specific gravity of the Fundulus egg in hypertonic salt solutions after exposure to the follicular extract give additional evidence of this permeability. 3. The permeability of the cells which are already a part of the developing organism can be apparently affected by the follicular extract, and these disturbances in the injected embryo were most marked in the head region. 4. The absence of a profound difference in the restoration of heart beats in the controlled and experimental embryos is probably due to the change in permeability in the epidermal layers preventing access of the follicular extracts to the deeper tissues. 5. There was no evidence for a stimulation of cell proliferation in the Fundulus embryo.

4710

Results Obtained From Applying the Feulgen Reaction to Protozoa.

MIRIAM SCOTT LUCAS. (Introduced by McKim Marriott.)

From the Anatomical Laboratory, Washington University School of Medicine.

Certain of the free-living and associated protozoa have been selected for a preliminary study of their reaction to the Feulgen thymonucleic acid test. They have been considered as to their status, source, nuclear organization and their reaction to this test. Extranuclear inclusions and organelles have received attention, as well.

Thymonucleic acid has been found to be present in some portion of the nuclear apparatus of each protozoan tested, thus far. Whether it can be said that the Feulgen reaction is indicative of the presence of chromatin is somewhat doubtful.

In *Paramecium multimicronucleatum* and *Paramecium aurelia,* both the macronucleus and micronuclei of each species gave a decidedly brilliant purple color reaction, while in the "associated" protozoan, *Balantidium coli* from the guinea pig, the macronucleus was a brilliant purple and the micronucleus a pale violet or negative. Presumably the micronuclei play similar rôles in the life-history of the two ciliates. Division stages of these two forms have not yet been secured for use in this reaction.

The food vacuoles of Paramecium contain bacteria and other ingested food particles. These vacuoles gave a faint violet reaction, which was due doubtless to the presence of the bacteria. The remainder of the protozoan was colorless.

The presence of host intestinal epithelial cells within the endoplasm of Balantidium was definitely established. The nuclei of these host epithelial cells gave the same reaction within the protozoan body as did those nuclei *in situ,* in the same field of the microscope.

The macronucleus of *Balantidium coli* showed vesicular areas similar to those previously described by the author[1] for the same species. In the Feulgen preparations these areas appeared as clear colorless vesicles, as they did when stained with alum cochineal, in contrast to their intense dark staining in Heidenbain's Iron Haematoxylin.

In the Infusoria there is generally considered to be a qualitative division of nuclear material into (1) a vegetative nucleus (macronucleus) which during division of the protozoan divides by amitosis and (2) one or more generative nuclei (micronuclei) which divide by mitosis.[2] It is interesting to note that the macronucleus in each of these ciliates contains an abundance of thymonucleic acid as indicated by the Feulgen reaction.

Giardia lamblia has been studied only in the encysted stage, with respect to this reaction. Results obtained are similar to those of da Cunha.[3] Binucleate and quadrinucleate cysts showed brilliant purple karyosomes, under this treatment, with a clear perikaryosomal area. No other parts of the protozoan reacted to the test by any color production.

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[1] Scott, M. S., *J. Morph. and Physiol.,* 1927, xliv, 436, 441.

[2] Hegner, R. W., and Taliaferro, W. H., Human Protozoology. Macmillan, 1925, 11, 17.

[3] Cunha, A. M. da, and Muniz, J., *Compt. Rend. de la Soc. d. Biol.,* 1928, xcix, 1339.

Chaos diffluens showed[4] a coarse granular area in the nucleus, which gave a vivid purple color, with the remainder of the nucleus clearly colorless. This irregular central mass of chromatin, which may also be distinguished in Giemsa preparations, varied in size and shape. Frequently it occupied a relatively small area of the nucleus and stretched out lengthwise in the shape of a "w", and at other times appeared as a large irregular mass. We may judge from this that the thymonucleic acid of this nucleus is segregated into one mass of the nuclear material. All of the usually so-called chromatic material of the nucleus does not give a positive reaction therefore.

The food vacuoles of *Chaos diffluens* gave a faint violet reaction as did those of Paramecium, due doubtless to the ingested bacteria. An instance of cannibalism was observed, where a large individual of Chaos had completely ingested a Paramecium. The Paramecium nucleus gave the characteristic brilliant purple color reaction.

A multinucleate Opalina from the rectum of the frog also indicated a regional arrangement within each nucleus of the chromatic material which gave a purple color. No color appeared elsewhere in the protozoan. Several large brilliant purple masses appeared in peripheral distribution within each nucleus. The remainder of each nucleus was quite faint or entirely negative in reaction.

These data suggest the value of the Feulgen thymonucleic acid reaction applied to the protozoa.

4711

Condenser Technique for Measuring Glass Cell or Other Potentials in Circuits of High Resistance.

GEORGE H. BISHOP.

From the Department of Physiology, Washington University School of Medicine, St. Louis.

Glass cell potentials or other electrode potentials in series with high resistance can be measured conveniently by a modification of Beans and Oaks'[1] condenser technique. Instead of the simple ballistic method employed by them, the cell is compensated by a potentio-

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[4] Schaeffer, A. A., *Carnegie Inst. Publ.*, 345, 1926, xxiv.

[1] Beans, H. T., and Oaks, E. T., *J. Am. Chem. Soc.*, 1920, xlii, 2116.

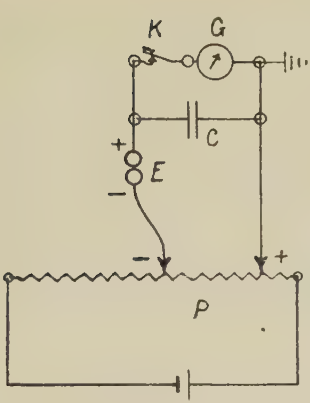

Figure 1.

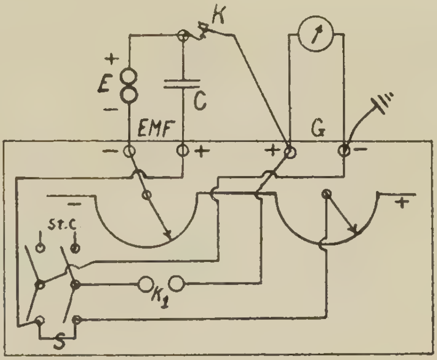

Figure 2.

FIG. 1.

Glass-cell potentials determined by the aid of a condenser. The uncompensated difference of potential between the potentiometer *P* and cell *E* slowly charges the condenser *C*, which is then suddenly discharged through *K-G*. The potentiometer is now readjusted until a null point is indicated.

FIG. 2.

Adaptation of the Leeds and Northrup Type K potentiometer to the scheme of Fig. 1. See text.

meter, and a null point reading made, thereby reducing polarization of the cell by reducing the charge on the condenser (Fig. 1).

The Leeds & Northrup type K potentiometer lends itself readily to such an arrangement. Glass cell and condenser are connected in series to the regular E.M.F. binding posts (Fig. 2). From a point between glass cell and condenser a lead passes to the insulated lower contact of the key *K* and from the upper contact to the galvanometer binding post proximal to the E.M.F. posts. The double point, double throw switch of the potentiometer is short-circuited across its front posts, at *S*, and the potentiometer may still be checked against the standard cell in the usual manner without complication. The potentiometer tap keys with resistance protection (K_1) are employed as usual in reading the standard cell check, and the extra tap key *K* only is used in reading the glass cell-potentiometer balance, the condenser acting as a protection to the galvanometer.

A 2 m.f. condenser is sufficient to give a reading to 0.2 millivolt on a Leeds & Northrup high sensitivity galvanometer (10^{-10} amp./mm.) critically damped by a shunt. With a glass cell of 150 megohms resistance, the time of charge to 1/2 final voltage of the condenser through this resistance is 3.5 minutes, the time to 90% is 12 minutes. Adjustment to within one millivolt of the null point can be made by readings at intervals of only a few seconds, since the condenser need be charged to only a small fraction of its full capacity to obtain this sensitivity. The system is grounded at the galvanometer and requires no shielding; careful insulation is required only for the glass cell itself, the condenser and the lower key contact. These are mounted on paraffin. 2 mf. by-pass condensers from radio stocks can be selected, which have a resistance to direct current of over 10^{11} ohms, or over one thousand times that of average glass cells.

This arrangement is also satisfactory for reading micro-electrodes or potentials in any high-resistance circuit, the value of the condenser being chosen to give the required sensitivity.

4712

Plasma Protein, Erythrocyte Sedimentation and Serum Lability in Tuberculous Individuals.

L. R. JONES. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Sedimentation of the corpuscles of heparinized blood was determined by noting the extent of fall in glass tubes with an inside diameter of 4 mm. and height of 100 mm. during an interval of 1 hour. The tubes were centrifuged to insure complete sedimentation and the sedimentation-index computed as the ratio between the sedimentation observed in 1 hour and the possible maximum extent of sedimentation.

Precipitability of serum protein was determined by adding various amounts of aluminum sulphate, as contained in a unit volume of 1 cc. to 0.2 cc. of unheated blood serum in small tubes. Serum and reagent were mixed and set aside at room temperature for 1½ hours. A heavy flocculent precipitate that settled out leaving a clear supernatant fluid was recorded as a positive reaction.

Average values for these blood properties as observed in a group of 14 tuberculous and a group of 20 normal individuals are listed in the accompanying table with average values for total protein, fibrin, globulin and albumin as contained in the plasma.

In the presence of tuberculous infection the blood of man exhibits a quantitative shift in plasma protein toward the more labile globulin and fibrinogen fractions, also an increased sedimentability of the corpuscles and an increased precipitability of serum protein. The latter changes are coincident with but not necessarily related to changes in plasma protein. Apparently with the inception of a

Plasma—100 cc.

	20 Normal Male Individuals	14 Tuberculous Male Individuals
Total protein gm.	6.95	7.51
Fibrin gm.	0.26	0.43
Globulin gm.	2.05	2.94
Albumin gm.	4.64	4.11
Blood Sedimentation-Index	12.5	66.4
Incidence of minimal precipitation with alum. sulph. per cent		
0.01	0	0
0.02	0	2
0.03	0	4
0.04	0	6
0.05	0	2
0.06	6	all
0.07	7	all
0.08	2	all
0.09	5	all
0.10	all	all

general biologic reaction in the course of tuberculous disease, a decrease in blood stability (as measured by these properties) becomes apparent and is quantitatively without relationship to the extent of tuberculous involvement.

Clinical and experimental value of the aluminum sulphate flocculation test may be extended by the titration procedure outlined.

4713

Can Bacteriophage Be Detached From the Carrier-Particles?

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From the Department of Bacteriology and Immunology, Washington University Medical School.

It has been found earlier that residue remaining on the membrane after ultrafiltration of bacteriophage through collodion retains a large proportion of the active agent and does not give it off freely into the filtrate on repeated washing with water. But, if broth is substituted for water in washing, the active agent reappears in the filtrate in high concentration.[1]

This finding suggested at first that passage of broth through the membrane resulted in coating the filtering bed by the colloids present in the broth, thus rendering it permeable for coarser particles which were held up by the membrane when suspended in water. However, further experiments have shown that preliminary passage of broth through a new membrane similar to that used above failed to increase its permeability to the residual fraction of the phage. On the basis of these findings the results of the earlier experiments were interpreted as indicating that the addition of broth directly to the residue caused detachment (elution) of some of the active agent from the coarser particles and its adsorption on and passage with the finer particles of the broth.

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[1] Bronfenbrenner, J., *J. Exp. Med.*, 1927, xlv, 873.

The existence of a method[2] permitting the measurement of the particles which carry phage suggested the means of inquiring into the validity of this tentative conclusion. We determined by this method the size of ordinary broth-phage filtered through Berkefeld filter as being in the average 12 mμ in diameter.[2] Using the same procedure, we now find that particles held back by the membrane after ultrafiltration measure 28mμ in diameter (average). When broth (free from phage) is added to this residue and ultrafiltration repeated, the particles which come through measure 20mμ in diameter (average)—that is, they are smaller than the original particles kept back by the membrane.

All measurements in each of the experiments were carried out in triplicate and the differences recorded, while not very great, seem to be uniformly reproducible (irrespective of the differences in diffusion constants of the different porous plates used) and well outside of the limits of experimental error of the method.

The fact that the average size of the particles carrying phage through the ultrafilter upon the addition of broth to the residue (20mμ) falls just about midway between the average sizes of the particles of broth itself (12mμ) and that of the residue (28mμ) indicated that while some of the coarse particles of the residue may have been carried through as such, at least enough phage has been detached from the coarse particles of the residue and adsorbed on the smaller particles of the broth to influence measurably the average size of particles in the ultrafiltrate.

The significance of these findings lies in the fact that they show that the particles carrying the active principle are not autonomous but that bacteriophage is merely adsorbed on them, and under favorable conditions can be detached from the original carrier particles.

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[2] Hetler, D., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 644.

Pacific Coast Section.

University of California College of Medicine, December 11, 1929.

4714

Effect of Sodium Amytal on the Hyperglycemia Due to Morphine.

SELLING BRILL. (Introduced by C. D. Leake.)

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Sodium amytal (sodium isoamylethyl barbiturate) was introduced by Page[1] as an anesthetic without influence on blood sugar. He has been supported in this claim by competent observers[2] although Weiss and others[3] disagree with him. It has been stated by some physiologists that unless an animal was under sodium amytal anesthesia, blood sugar figures could not be accepted as being uninfluenced by the emotional state of the animal.

In this connection it is interesting to note the effect of amytal anesthesia on the hyperglycemia induced in dogs by morphine sulphate. In Table I are given blood sugar figures on 7 dogs. Two series of blood sugar determinations, several days apart, were made on each animal; first, following the injection of morphine sulphate alone and secondly, following morphine injection with the animal under amytal anesthesia. The dogs varied in weight from 7 to 18 kilograms. They were on a routine diet and were last fed 14 to 18 hours before the beginning of the experiment. The dose of morphine sulphate was 10 mgm. per kilo body weight given subcutaneously and the dose of sodium amytal was 50 mgm. per kilo intraperitoneally. Blood samples were taken from a vein on the hind leg and sugar determinations were made by the method of Hagedorn.[4]

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[1] Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 194.

[2] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 127. Hepburn, J., *et al.*, *Am. J. Physiol.*, 1924, lxix, 563. Edwards, D. S., and Page, I. H., *Am. J. Physiol.*, 1924, lxix, 177.

[3] Weiss, S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 363. Underhill, F. P., and Sprunt, D. H., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 137.

The results indicate that sodium amytal anesthesia in dogs has a marked inhibiting effect on the hyperglycemia due to morphine.

TABLE I.

Experiment.	Blood Sugar, mgm. per 100 cc.					
	During fasting	After 30 min.	After 1 hr.	After 1½ hrs.	After 2 hrs.	After 4 hrs.
I						
Morph. sulph. alone	94	162	212	212	202	147
Sodium amytal* + morph. sulph.	95	116	107	98	105	
II						
Morph. sulph. alone	86	154	212	221	207	164
Sodium amytal* + morph. sulph.	95	106	100	86	88	97
III						
Morph. sulph. alone	90	136	152	170	165	
Sodium amytal* + morph. sulph.	99	147	150	129	128	
IV						
Morph. sulph. alone	88	174	244	234	250	
Sodium amytal* + morph. sulph.	101	147	175	154	138	
V						
Morph. sulph. alone	83	165	216	200	169	164
Sodium amytal* + morph. sulph.	80	124	73	103	97	87
VI						
Morph. sulph. alone	84	95	133	135	142	121
Sodium amytal* + morph. sulph.	92	109	97	90	97	
VII						
Morph. sulph. alone	81	132	164	164	166	111
Sodium amytal* + morph. sulph.	97	91	98	100	105	112
Average						
Morph. sulph. alone	86.6	145.4	190.4	191.0	186.0	
Sodium amytal* + morph. sulph.	94.0	120.0	114.3	108.6	108.3	

* The sodium amytal injection preceded the morphine injection by about 20 minutes.

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[4] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, cxxxv, 46.

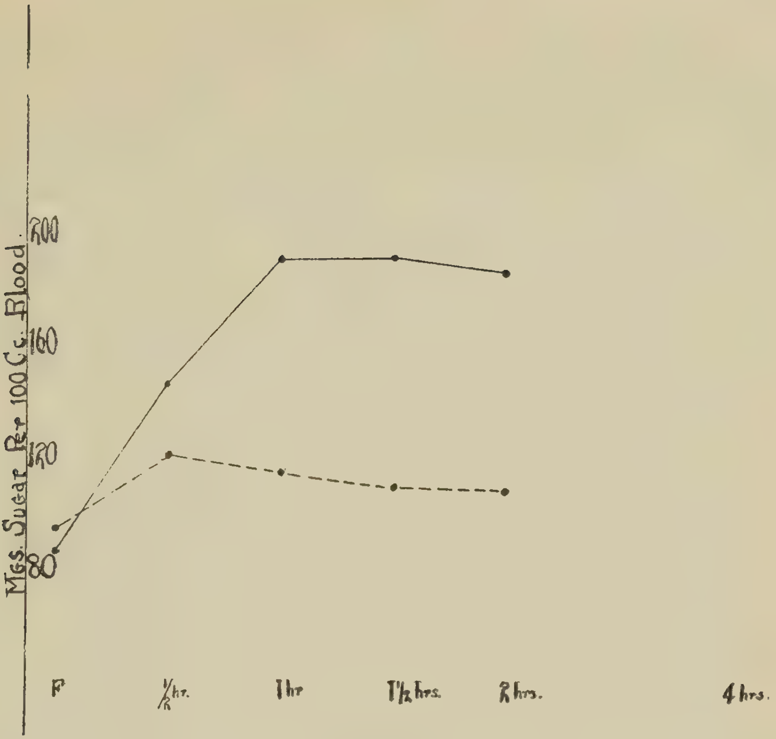

CHART I.
The unbroken line is the average composite curve of the blood sugar determinations on seven dogs following the injection of 10 mgm. morphine sulphate per kilo; the broken line, the composite curve on the same animals under amytal anesthesia and morphine.

4715

Toxicity of Acetarsone (Stovarsol) and its Calcium and Sodium Salts on Oral Administration to Rabbits and Cats.*

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From the Pharmacological Laboratory of the University of California Medical School, San Francisco.

Acetarsone (acetyl-amino-hydroxy-phenyl arsonic acid) was synthesized in 1921 by Tréufouel and Fourneau[1] after having been prepared earlier by Ehrlich.[2] Levaditi[3] noted the drug was toxic for rabbits in doses of 0.66 gm. per kilo on oral administration, and that it was tolerated in doses of 0.3 to 0.4 gm. per kilo. Since then the drug has been recommended for syphilis, amebiasis, and other protozoan infestations. Conflicting reports have appeared on its therapeutic efficiency and toxicity. Pool,[4] Worms,[5] and Kolle[6] found this compound more toxic than Levaditi originally reported, causing death in rabbits when 0.2 to 0.3 gm. per kilo were given by mouth.

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* This report is based on part of an extended cooperative study of the chemotherapy of amebiasis conducted by the Department of Pharmacology of the University of California Medical School and the Pacific Institute of Tropical Medicine, San Francisco.

† Lilly Research Fellow.

[1] Fourneau, E., *Ann. Inst. Pasteur*, 1921, xxxv, 571.

Levaditi and Poole worked on material synthesized by Fourneau. We attempted to find the minimal lethal dose on oral administration to cats and rabbits of the commercially available product, and have also studied the toxicity of its calcium and sodium salts.[7]

The material was powdered and administered in gelatin capsules washed down with water. It was necessary to anesthetize the cats for this procedure. All animals were kept in individual cages, under identical conditions of diet and hygiene, and were observed for at least 30 days following the administration unless death intervened. Those animals dying within the observation period were subjected to post-mortem examination and sections of the principal organs were taken for histological study. Untreated animals were maintained in the laboratory under similar conditions as controls.

Table I shows the results of this study on 35 cats and 56 rabbits. The determination of the minimal lethal dose on oral administration is difficult because of the uncertainty of absorption. The range of toxicity of these compounds indicated by our figures seems reliable except in the case of calcium acetarsone in rabbits. Poole noted that some animals survived very high doses of sodium acetarsone.

In lethal doses animals lost weight markedly. Lethargy, anorexia, abdominal distension, and soft stools were noted. Immediately before death the pupils became constricted, respiration was increased, the abdomen bloated, the muscles relaxed, and on stimulation clonic convulsions were observed.

In animals dying from lethal doses necropsy revealed distension of the stomach, large intestines, and bladder, congestion of the lungs,

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[2] Ehrlich, P., and Hata, S., *Die experimentelle Chemotherapie de Spirollosen*, Berlin, J. Springer, 1910.

[3] Levaditi, C., *et al.*, *Ann. Inst. Pasteur*, 1922, xxxvi, 729.

[4] Poole, A. K., *Johns Hopkins Hosp. Bull.*, 1926, xxxviii, 242.

[5] Worms, W., *Deutsche Med. Woch.*, 1925, li, 428.

[6] Kolle, W., *Deutsche Med. Woch.*, 1924, i, 1074.

[7] Supplied by Lilly Research Laboratories.

TABLE I.
Toxicity of Acetarsone for Rabbits and Cats on Oral Administration.

Animal and M.L.D.	Dose in mgm./kg.	No. used	No. lived	No. died	Time of death
					days
ACETARSONE Arsenic content about 27.2%					
Rabbits	100	2	2	0	—
	125	5	3	2	10, 11
125-150 mgm./kg.	150	5	2	3	9, 9, 13
5/10 died	175	5	3	2	4, 12
	200	5	1	4	10, 12, 19, 20
	225	2	1	1	26
Cats	100	2	2	0	—
	125	2	2	0	—
150-175 mgm./kg.	150	4	3	1	3
3/6 died	175	2	0	2	3, 20
	200	3	0	3	10, 20, 29
	250	1	0	1	8
CALCIUM ACETARSONE Arsenic content about 23.8%					
Rabbits	75	2	2	0	—
	100	4	2	2	6, 8
Not determined	125	2	1	1	10
	150	5	4	1	9
	200	3	1	2	8, 14
	250	1	1	0	—
Cats	125	2	2	0	—
	135	3	2	1	3
135-150 mgm./kg.	150	5	2	3	3, 3, 5
4/8 died	200	1	0	1	3
SODIUM ACETARSONE Arsenic content about 25.3%					
Rabbits	75	1	1	0	—
	100	2	2	0	—
150 mgm./kg.	125	5	4	1	14
2/4 died	150	4	2	2	7, 14
	200	2	0	2	7, 7
	250	1	0	1	7
Cats	100	2	2	0	—
	125	4	1	3	3, 4, 30
125-150 mgm./kg.	150	3	1	2	3, 22
5/7 died	200	1	0	1	3

liver, and kidneys, and occasional hemorrhages in the kidneys. Microscopic sections[8] showed congestion of the lungs, liver, spleen, and kidneys, with deposition of pigment in these organs. Tubular degeneration of the kidneys with occasional hemorrhages was seen in several sections. Moderate granulation of cardiac fibres was noted. Disorganization of the muscle fibres of the stomach was observed. The liver cells were filled with fat globules. Our findings agree with those of Heitzmann,[9] who also mentions blood cell destruction, epithelial necrosis, calcification in the kidneys, and fatty deposits in the heart, liver and kidneys.

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[8] Through the courtesy of the Department of Pathology, University of California Medical School.

[9] Heitzmann, O., *Archiv. f. Derm. u. Syph.*, 1926, clii, 344.

Summary: The minimal lethal dose of commercially available acetarsone administered orally to cats and rabbits is between 125 and 175 mgm. per kg. This is about 4 times the toxicity originally reported by Levaditi for the material prepared by Fourneau. In view of the relatively large number of clinical cases of acetarsone poisoning these experimental findings indicate the necessity of a revision of the recommended therapeutic dose.

The minimal lethal dose of the calcium and sodium salts of acetarsone on oral administration to cats and rabbits seems to be about the same as that of acetarsone.

4716

Osseous Changes Due to Pressure Trauma.

JOHN ALBERT MARSHALL.

From the Geo. Williams Hooper Foundation for Medical Research and the College of Dentistry, University of California, San Francisco.

When a continuous pressure is maintained upon the bone in the alveolar process of the maxilla the resulting trauma is characterized by several histo-pathological changes.

The manner of applying pressure is by means of bands cemented upon the crowns of the teeth. To these spring wire has been previously soldered so that when the appliance is in place the tension may be increased, decreased or changed in direction merely by bending the spring wire. Such appliances have been in place on teeth of monkeys *(Macacus rhesus)* from one day to 8 months and the results have been so striking that this preliminary report seems appropriate.

When the crowns of teeth are separated distally their roots are forced nearer each other. This pressure produces areas of absorption in the alveolar process as well as on the periphery of the roots. Combined with this absorption is a great amount of fibroblastic activity. These changes commence apparently early in the experiment as shown by the examination of the tissues from an animal on which the appliances had been in place only 24 hours.

In one case, where the greatest possible tension was applied in the effort to produce absorption, the alveolar process between the teeth was fractured.

The force used in producing this result as measured by Jolly balances was found to be one and three-quarters (1¾) pounds.

Approximating teeth were subjected to elongation tension and its opposite a depression tension. This double pull stretched the supporting tissues to a great degree and the effect was one in which the bone at the alveolar crest, the overlying connective tissue and mucosa have been pulled or dragged out of their proper position and have followed the movement of the attached tooth.

At the apex of the tooth the space between it and the bone is filled normally with a few layers of peridental membrane. The tooth which was pulled gradually from its socket showed a different picture. In this case the amount of peridental membrane had increased to 5 or 6 times its usual thickness.

The apex of the tooth which was being forced through the end of the socket was deeply eroded.

The fibres of the dentin matrix also show changes. They do not branch in the usual manner near the junction of dentin and cementum; they are smaller in number and in size and appear to be attenuated at the ends. The condition resembles somewhat the appearance of the fibres under an area of caries in which secondary dentin is being deposited.

When comparison is made between the processes concerned with the radicular resorption of deciduous teeth, it is at once apparent that differences are clearly manifest. There are even more striking dissimilarities demonstrable when bone absorption resulting from apical infection is studied.

In this case a low grade chronic infection may develop into an acute abscess. But in neither case has it been found possible to increase experimentally the rate of bone or tooth absorption.

4717

Studies on the Combination of Iron With Certain Proteins, Amino Acids and Related Compounds.*

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From the Division of Biochemistry, University of California Medical School, Berkeley.

The present work is a continuation of the studies which have been carried out in this laboratory on the mode of combination of proteins with the inorganic elements. Previous reports have dealt with the compounds of proteins with the alkali metals[1] and with the alkaline earth elements.[2] This work has now been extended to include the compounds which ferric iron forms with certain proteins, amino acids and related substances.

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* Aided by a grant from the Chemical Foundation, Inc.

The method employed consists in adding the substances to be tested to a known solution of ferric iron. To this a standard amount of ammonium thiocyanate is added and the reaction is adjusted to a definite pH. The color of this solution is compared with a standard iron thiocyanate solution. If the substance tested forms a compound with iron (*e. g.*, complex ions) which is less dissociated than ferric thiocyanate, the color of the solution will be less intense than the standard. If the ferric compound is dissociated to a greater extent than ferric thiocyanate, no appreciable effect on the color will be noted.

The results showed that at pH 2.5 substances which possess a hydroxyl group alpha to a carboxyl group markedly reduce the color of ferric thiocyanate. The effect is less pronounced when the hydroxyl group is in the beta position. Dicarboxylic acids, which may be considered as hydroxy acids, show some effect. Hydroxy acids which contain a double bond oxygen (=0) and hydroxy groups in an arrangement such as occurs in phosphoric acid, arsenic acid and sulfuric acid markedly reduce the color of ferric thiocyanate. Casein and gelatin likewise exhibit this property. Substances which contain only hydroxyl groups (glycerine, borates, etc.) showed within the limits of concentration tested, no effect. The mono-amino, mono-carboxylic amino acids (glycocoll, alanine, δ-amino valeric acid) and the diamino mono-carboxylic amino acid (lysine) exhibit no effect.

Starkenstein[3] has recently published data from which he concludes that a great many substances combine with ferric iron to form complex compounds.

The present work is being continued.

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[1] Greenberg, D. M., and Schmidt, C. L. A., *J. Gen. Physiol.*, 1924, vii, 287.

[2] Greenberg, D. M., and Schmidt, C. L. A., *J. Gen. Physiol.*, 1926, viii, 271.

[3] Starkenstein, E., *Z. Ges. Exp. Med.*, 1929, lxviii, 425.

4718

Circulatory Actions of Digitalis and Strophanthus and Comparisons With Histamine and Epinephrine.

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In a previous study[1] of the circulatory actions of digitalis in dogs we showed that the diminution in cardiac output was dependent upon the lowering of venous pressure, rather than upon cardiac depression, *i. e.,* that it was of vascular and not cardiac origin. It was suggested that this lowering of venous pressure from digitalis was mediated by a hepatic vein constriction with pooling of blood in the portal vessels. In the present study we have amplified the evidence and confirmed the importance of the hepatic mechanism previously suggested.

It was found that intravenous injections of digitalis, in doses corresponding to the full therapeutic for man, and of strophanthus, in 5 dogs caused an increase in arterial pressure, fall of venous (intraauricular) pressure, and increase in portal vein pressure. The increase in portal vein pressure was due to hepatic vein constriction, agreeing with the increased liver volume previously observed by us. The latter actions agree also with similar actions on isolated hepatic veins and on those of perfused livers reported in the literature.

After elimination of the liver (6 dogs) from the circulation, by shunting the portal blood into the vena cava, or of the entire splanchnic circulation by ligation, digitalis and strophanthus did not diminish the venous pressure, but increased it, if anything. Accordingly, the fall of venous pressure, after digitalis and strophanthus in dogs with liver intact, was due to diminished venous flow owing to pooling of blood in the splanchnic (mainly portal) region as a result of block in the liver. This mechanism adequately explains the diminution in cardiac output after digitalis, since a diminished venous return must result in deficient cardiac filling and output.

Comparisons were made with known actions of histamine and epinephrine in the same animals. Comparable pooling of portal blood was observed, although modified by the other vascular actions of these drugs.

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[1] Dock, W., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 524.

4719

The Actions of Synthetic Salicyluric Acid.

P. J. HANZLIK AND M. A. SEIDENFELD.

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The occurrence of a natural salicyluric acid in the body, and its significance as a detoxification product, after administration of salicylates, analogous to hippuric acid after benzoates, have been much discussed but not conclusively settled. Owing to previous negative results by one of us[1] with chemical methods of isolation from urines, it was attempted to obtain indirect evidence on the question by a study of the pharmacological actions of synthetic salicyluric acid. The pure compound was synthesized according to E. Fischer's method[2] by Dr. Robert T. Dillon of the California Institute of Technology, Pasadena, to whom our thanks are due.*

It was found that synthetic salicyluric acid was comparatively stable *in vitro,* since it was not hydrolyzed in incubated buffer-salt solutions with hydrion concentrations falling within the range of body fluids, and in these solutions containing ferments, yeast and bile-salts. Some hydrolysis occurred in solutions of pH 2 and 14 and complete hydrolysis in strong acids and alkalies.

On the other hand, the compound suffered marked hydrolysis in its passage through the body, since from one-half to three-fourths of the total excreted salicyl in urines of human subjects (6) consisted of ordinary salicylate. The total salicyl excretion was about 88% of the total administered (from 4 to 28 gm.). The effects on urinary nitrogenous metabolites were variable or insignificant. The symptoms of salicylism in human subjects were weak, even with the highest oral doses, but in white mice, white rats, pigeons and a dog (total, 89 animals) typical symptoms of salicyl poisoning were demonstrated after hypodermic and intravenous administrations. The M.F.D. in the majority or 60% of the mice was 1.13 gm., and in rats 3 gm., per kilo. The toxicity in mice was proportional to the amount of liberated salicylate, assumed from the results on urinary partition, and corroborated by the actual toxic dose of salicylate.

Accordingly, the results obtained indicate that significant quantities of salicyluric acid can not be expected in the body after administration of salicylates, and therefore, the alleged biochemical rôle of the compound is not sustained. This agrees with the negative results of chemical isolation, and also with the vast majority of results on excretion which show that the salicylates are excreted mainly unchanged.

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[1] Hanzlik, P. J., *J. Pharm. Exp. Therap.*, 1917, x, 461.

[2] Fischer, E., Ber., 1909, xlii, 215.

* The cost of materials was defrayed by grants from the Committee on Research of Stanford University.

4720

Duck Disease Studies. III. Salt Content of Soils in Disease and Non-disease Areas.*

PAUL A. SHAW. (Introduced by C. D. Leake.)

From the Hooper Foundation for Medical Research of the University of California, San Francisco.

It has been shown[1] that oral administrations of certain inorganic salts, alone or in combination, are definitely toxic for ducks. The greatest toxicity in these experiments was found in sodium chloride and sodium sulphate mixtures containing in addition either magnesium or bicarbonate *together with nitrate ions.* The symptoms exhibited by the birds after feeding with these mixtures resembled in many respects those noted in "duck disease". The salt composition of soils from disease and non-disease areas was studied to attempt to correlate these experimental findings with the naturally occurring condition.

Soil samples were obtained from the following areas:

A. Disease Areas. 1. San Joaquin Valley (West of Pond, Calif.). 2. Tule Lake (Modoc County, Calif.). 3. Government Sump (South of Klamath Falls, Ore.).

B. Non-disease Areas. 1. San Joaquin Valley (Los Banos area). 2. Isolated ponds south of Klamath Falls, Ore.).

All samples were taken near the surface of the soil and within a few yards of the water's edge. Analyses were made on water extracts. Carbonate, bicarbonate, and chloride were determined by titration. Calcium, magnesium, and sulphate were approximated

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* Part of an extended cooperative study between the California Fish and Game Commission, the Hooper Foundation for Medical Research of the University of California, and the Department of Pharmacology of the University of California Medical School, San Francisco.

[1] Shaw, P. A., Proc. Soc. Exp. Biol. and Med., 1929, xxvii, 6.

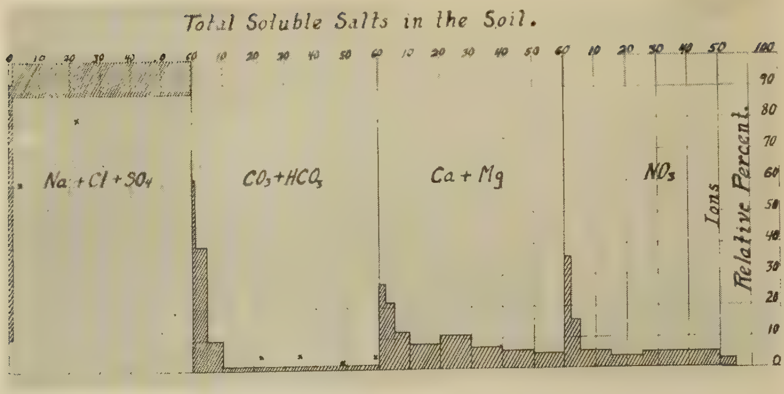

FIG. 1.

Diagram showing relative percentages of the different groups of ions in relation to total soluble salts in the soil.

(after precipitation) by comparative turbidity tests. Nitrate was determined by aluminum reduction followed by distillation and titration. Qualitative tests only were made for nitrite. Sodium was computed by difference in the reaction value of basic and acidic ions. The ions were grouped for study as follows: 1. Chloride, sulphate, and sodium. 2. Carbonate and bicarbonate. 3. Calcium and magnesium. 4. Nitrate.

In Fig. 1 are shown the relative per cents of the different groups of ions in relation to increasing amounts of salts in the soil. This chart includes 50 samples from all areas and is presented for the purpose of showing the general character of the soils studied. Above 2% total solubility, chloride, sulphate, and sodium ions comprised 89% or more of the soluble salts except in 2 instances. Below 2% solubility the relative amount of these ions fell to a minimum of 11%. This indicates, in the areas studied, that the major increase in total salts is composed of sodium chloride and sodium sulphate.

The charts for the 3 remaining groups of ions indicate that the highest values are found in waters or soils of low alkali content, the relative amounts rapidly decreasing for higher alkali concentrations. In many samples one or more of these groups were entirely absent, which is indicated on the chart by the shaded areas extending to the base line.

The chief point of interest is shown in Table 1, and graphically in Fig. 2. The data is divided in two portions; one showing analyses of 7 soils collected in the 2 non-disease areas; the other, of

TABLE I.

No.	Mgm. per 10 gm. of soil.				
	Total	Cl, SO_4, Na	NO_3	Ca, Mg	CO_3, HCO_3
Non-disease Areas.					
1	1076	1050	4	11	11
2	2168	2140	2	24	2
3	2304	2060	12	230	2
4	3398	3110	0	258	30
5	4386	4110	0	232	44
6	5550	5495	0	0	55
7	6294	5750	0	302	242
Disease Areas.					
1	2268	2240	2	25	1
2	2346	1910	84	244	108
3	2410	2280	63	53	14
4	2604	2464	84	51	5
5	3293	2937	112	224	20
6	3355	3000	77	214	64
7	3688	3441	184	59	4
8	3727	3430	37	242	18
9	4732	4200	250	160	122
10	4941	4704	109	59	69
11	5508	5000	95	264	149

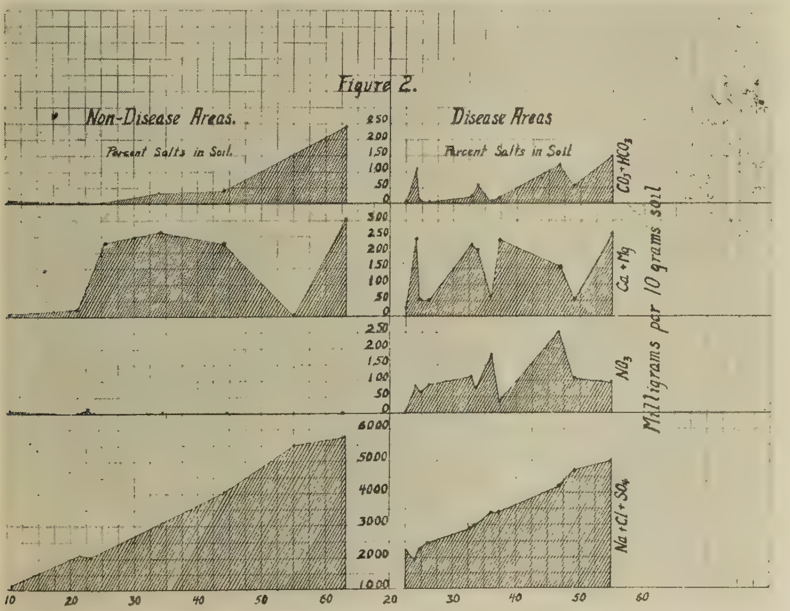

FIG. 2.

Diagram showing mgm. of the different groups of ions per 10 gm. of soil from disease and non-disease areas in relation to total soluble salts.

11 soils from the 3 disease areas. Only soils of comparatively high alkali concentration have been shown. In Fig. 2, mgm. of each group of ions per 10 gm. of soil are plotted against per cent total soluble salts.

The amounts of nitrate ions from disease and non-disease areas alone show a marked difference, being practically zero in non-disease areas studied, while from 50-250 mgm. per 10 gm. of soil were found in all but 2 of 11 samples from the 3 disease areas examined. This observation is particularly significant in view of the marked toxicity noted in ducks as a result of artificial feeding with salt mixtures of sodium chloride and sodium sulphate, containing nitrate ions and also magnesium or bicarbonate ions.

Two additional factors indicate that nitrate may play an important rôle in the incidence of "duck disease". Sodium nitrate is several times more soluble than the corresponding chloride and sulphate. One may therefore expect nitrate to become an appreciable factor in the surface alkali only at the hottest and driest time of the year. In addition, nitrate is also increased at this time through bacterial decomposition of organic matter. These considerations correspond to the usual incidence of the duck malady in late summer and fall.

4721

An Experimental Method for the Radiographic Demonstration of the Bronchial and Pulmonary Arteries.

MARY E. MATHES, F. L. REICHERT AND EMILE HOLMAN.

From the Laboratory of Surgical Research, Stanford University Medical School.

In a previously described series of experiments[1] it was found that the introduction into the venous circulation of an embolus infected with pyogenic organisms may be followed by widely differing pathological changes in the parenchyma of the lung. To determine the exact conditions present within the pulmonary and bronchial circulations following the introduction of such emboli a method for the radiographic demonstration of the two circulations was developed.

Anatomically the bronchial arterial system usually arises as one or more branches from the first portion of the descending aorta anteriorly. Occasionally branches may arise from the first or second intercostal, the internal mammary, or the right subclavian arteries. They enter the lung at the hilum and course along the bronchi and their branches up to the *ductus alveolaris.*[2]

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[1] Holman, E., Chandler, L. R., and Cooley, C. L., *Surg. Gyn. and Obs.*, 1927, xliv, 328.

The bronchial artery is best injected with the lung *in situ.* Under chloroform or ether anaesthesia the chest is opened avascularly by splitting the sternum exactly in the midline. Just before entering

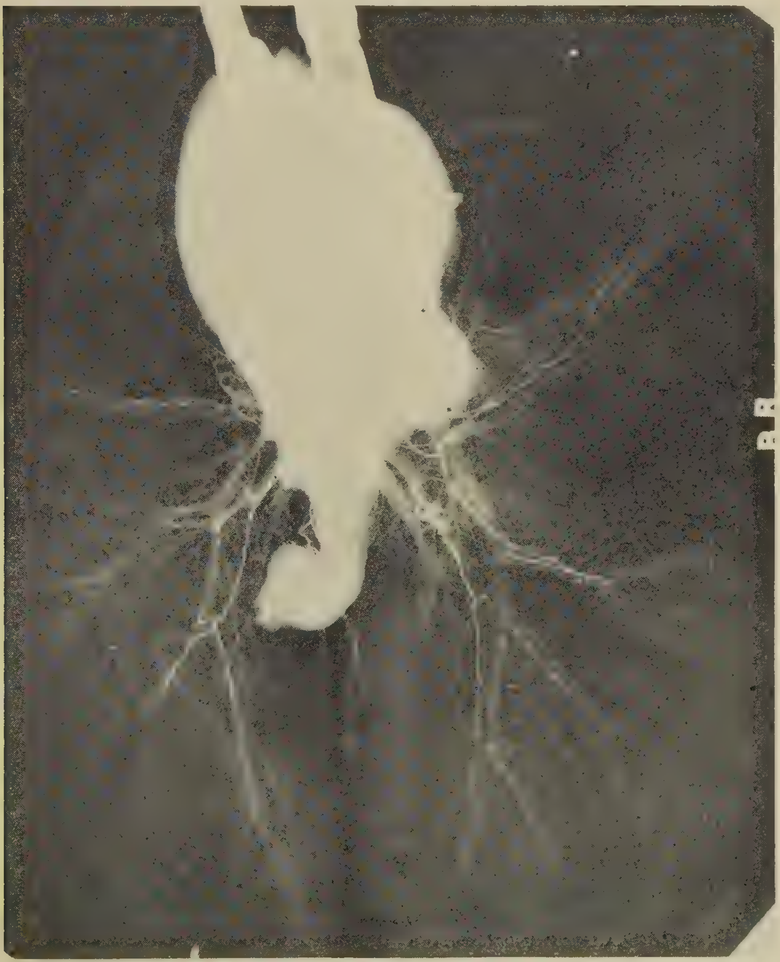

FIG. 1.
Roentgenogram of a normal lung after injection of the bronchial arterial system with Hill's bismuth mass.

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[2] Miller, Wm. Snow, *Am. Rev. Tbc.*, 1925, v, 87.

the pleural cavity the animal is killed with the anaesthetic to prevent injury to the inflated lung.

The innominate and left subclavian arteries are ligated as they leave the aortic arch. The first intercostal arteries should be clamped laterally to avoid occlusion of the bronchial artery which may arise from one of them. The thoracic aorta is tightly clamped distal to the fifth intercostal arteries so as to limit the injection to the thorax.

The pericardium is opened longitudinally and a large cannula inserted into the aorta through an incision in the left ventricle, all air is excluded from the system by filling the cannula and the attached tube with warm water before insertion into the ventricle. A heavy ligature passed around the base of both the aorta and pulmonary artery holds the cannula firmly with less danger of rupture of the aorta by cutting through of the ligature.

The most satisfactory injection mass is that of Hill[3] using a suspension of 20% bismuth oxychloride in an 11% solution of gum acacia. The bismuth mixture, preferably warm, must be repeatedly shaken to prevent settling. In our experiments Berlin blue has been added to distinguish the bronchial from the subsequent pulmonary arterial injection. The injection is begun by bringing the pressure immediately to about 160 mm. of mercury, which is the normal arterial pressure in the dog. To aid in complete filling of the bronchial vessels the lung is very gently manipulated during the injection, taking care to prevent injury to the pleura. Fifteen to twenty minutes are required to secure complete injection. The lungs and heart are removed by severing the vessels of the neck, the trachea, oesophagus and thoracic aorta. The intercostal vessels may be ligated and cut, but less trauma to the lung is produced when the intercostal vessels are carefully avulsed a centimeter or so distant from the aorta by passing the hand along the aorta dorsally. The vessels remain plugged with bismuth and no leakage occurs with careful handling.

After the lung is removed the intercostal arteries and the thoracic aorta are ligated, if the bronchial artery arises from the intercostal artery it may be torn in removing the lung and must be ligated.

A tube and cannula are tied into the trachea and the lung inflated so as to distend all atelectatic areas, care being taken never to inflate beyond a pressure of 40 mm. of mercury when the air sacs are ruptured. The lung is then deflated to an intrabronchial pressure of 10 mm. of mercury which is the optimum respiratory distention of the canine lung. Inflation of the lung and maintenance of normal pressure relations are points of greatest importance in any observations on the lung.

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[3] Hill, E. C., *Johns Hopkins Univ. Bull.*, 1929, xliv, 248.

Stereoscopic roentgenograms of the injected lung are made at this time. These enable one to study accurately the size and distribution of the bronchial artery.

After roentgenograms of the bronchial artery have been taken the pulmonary artery may be injected in the excised lung by inserting a cannula through the right ventricle into the pulmonary artery. Hill's mass is again injected this time at a pressure of 20-40 mm. of

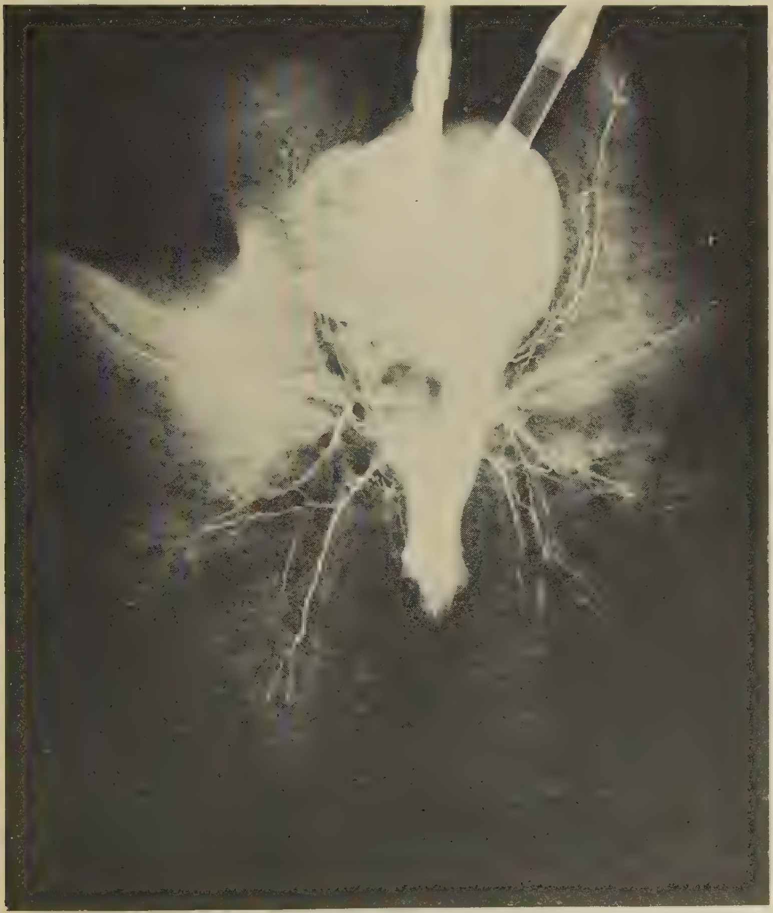

FIG. 2.
Roentgenogram showing a generalized dilatation of the bronchial arterial system in distemper pneumonia which involves the entire lung.

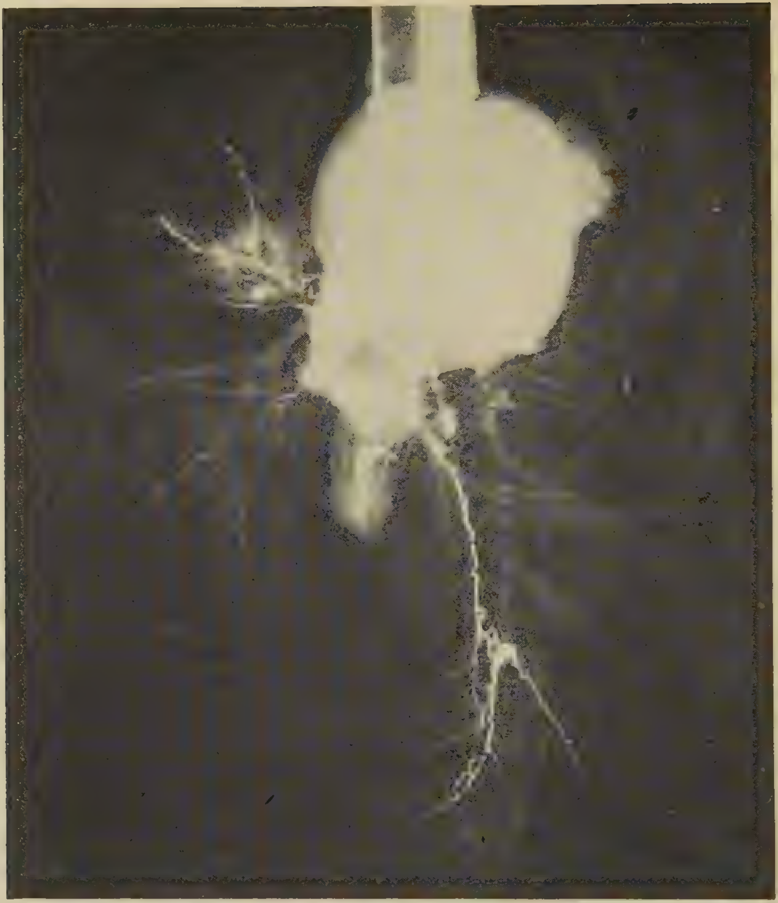

FIG. 3.

Roentgenogram demonstrating enlargement of the bronchial arteries to the lobes which contain emboli. The left upper lobe shows an abscess cavity while the right lower lobe contains a sterile embolus.

mercury, the lung being maintained in a state of inflation by an intrabronchial pressure of 10 mm. of mercury. Stereoscopic roentgenograms are made showing the relationship between the two arterial systems.

Injections are preferably made immediately after sacrificing the animal but may be successfully carried out from one day to several weeks after death if the animal is kept on ice. In such a case the chest should be repeatedly filled with warm water during the injection.

4722

The Penetration of Living Tissue by Ordinary Radiant Energy.

R. S. DANFORTH. (Introduced by C. D. Leake.)

From the Department of Pharmacology of the University of California Medical School, San Francisco.

Since water in a layer 10 millimeters or more thick is well known to be inpenetrable by ordinary radiant energy (that is, in the ultraviolet, visible, infra-red, and heat spectrum) of wave lengths longer than 1.4 micron, it has been suggested by Coblenz[1] that living tissue will, in general, be permeable only to wave lengths shorter than these. Sonne[2] found penetration of living tissue in his so-called "visible range", but his filters (water and 5% ferro-ammonium sulphate in glass chambers) apparently transmitted waves to a length of at least 1.3 micron.[3] On the other hand, Bachem and Reed[4] have recently shown that wave lengths shorter than 0.6 micron practically do not penetrate living skin. Evidence exists to show then, that the wave length range of ordinary radiant energy capable of penetrating living tissue lies between 0.6 and 1.4 microns, *i. e.,* it includes those infra-red rays lying near the red rays of the visible spectrum. Independent experiments initiated by me furnish further evidence in this regard, and indicate the extent to which these rays penetrate living tissue. The required measurements were made by G. H. Cartwright at the California Institute of Technology under the direction of R. C. Burt.

For convenience, the experiments on transmission were performed on one subject's cheek which averaged 5 mm. in thickness. Initial experiments indicated that this tissue transmitted about 14% of infra-red rays of a wave length of 0.86 micron. These experiments were made by means of a spectrograph and photographic plates. Opal glass 2 mm. thick was placed in front of the slit of the spectrograph to diffuse the light, the source of which was a 15 Watt electric light bulb. The extent of transmission was determined by comparing exposures made by this bulb placed 5 mm. in front of the diffusion glass, with exposures made by the bulb inside the mouth.

To determine the penetrability of living tissue over as great a range of radiant energy as could conveniently be measured, a specially sensitive vacuum thermocouple was constructed by G. H. Cartwright to replace the photographic plates. This thermocouple was mounted on a micrometer screw so that it could be moved across the spectrum with no significant change in the zero reading. Readings were made by galvanometer. The sensitivity of the thermocouple was such that energy of 1 x 10^{-8} calories per second striking the receiver changed the galvanometer reading by 1 microvolt. A light beam from a 500 Watt electric light bulb was concentrated by a lens and reflected from a mirror inside the mouth so that it passed through the cheek to the wide slit of the spectrometer.

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[1] Coblenz, W. W., *Trans. Illum. Eng. Soc.*, 1928, x, 252.

[2] Sonne, C., *Arch. Phys. Therap., X-Ray and Rad.*, 1929, x, 93.

[3] Nichols, E. F., *Phys. Rev.*. 1893, i, 12. Coblenz, W. W., *Bull. Bur. Stand.*, 1911, vii, 4.

[4] Bachem, A., and Reed, C. I., *Am. J. Physiol.*, 1929, xc, 604.

With this technique, the percentage penetration of radiant energy through the human cheek was found as shown in Fig. 1. This indicates a maximum transmission of about 21% at a wave length of 1.15 micron. Wave lengths longer than 1.5 micron or shorter than 0.54 micron showed no detectable transmission through the human cheek.

Sonne's work[2] indicates that in this range of the spectrum about 35% of the energy striking the skin is reflected. While no measure-

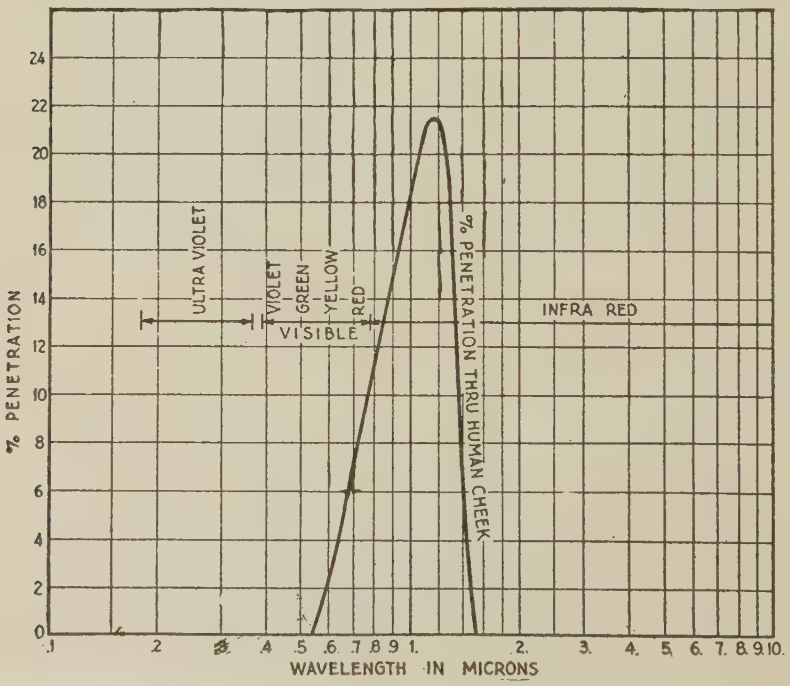

FIG. 1.
Diagram showing penetration of human cheek by ordinary radiant energy.

ments were made of energy reflected by the epithelium inside the cheek, it may still be assumed that about one-third of the energy entering the cheek passed through.

These studies indicate that living tissue is generally impermeable to all but a narrow band of ordinary radiant energy lying between wave lengths of 0.6 to 1.6 micron. Maximum penetration seems to occur at a wave length of 1.15 micron. What physiological or therapeutic effects such radiant energy has is still largely undetermined. A lamp specially designed to deliver rays in the range indicated has been made and is available for study.

4723

The Effect of X-Ray Upon Bacteriophage and Upon the Bacterial Organism.

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From the Departments of Bacteriology and Chemistry, University of California, Berkeley.

A number of workers recently have demonstrated that it is possible to produce variants among both plants and animals by exposure to the effect of X-rays We, in turn, have endeavored to demonstrate change by a similar procedure applied to the Bacteriophage and to the organism susceptible to the Bacteriophage, following suggestions made by Dr. Olson.

Seven strains of *B. coli* bacteriophage were selected and for each of these we cultured the particular strain of *B. coli* acted upon by its respective strain of bacteriophage. There were selected 7 coli bacteriophages and 6 strains of *B. coli* for this series of experiments. These bacteriophages were effective as follows upon the bacterial strains: one for all the strains, one for 5, one for 2 and 3 could lyse only one strain each of the organisms. There were thus 42 combinations possible in such a set up of tubes in case any variation were to appear.

The following methods for exposure were followed. The organisms were young cultures in the phase of positive logarithmic growth in beef infusion broth and the phages were recent filtrates in the same medium. These were contained within lead free glass test tubes. Exposure to X-ray was carried out at a distance of approximately fourteen inches from the target of a Roentgen tube actuated by approximately 75,000 volts of electricity. The exposure period was 30 minutes. Following this treatment each bacteriophage was placed in contact with untreated organisms of each culture and the exposed bacterial culture was set up with each of the unexposed bacteriophages. For control purposes a similar series of unexposed bacteriophages and bacterial cultures were used.

The following results were obtained. By this method of treatment 3 of the bacteriophages developed reduced effectiveness when brought into contact with organisms shown previously to be susceptible to them in the control series. But this reduction in lytic effectiveness does not of necessity appear when this same portion of X-rayed bacteriophage is brought into contact with other strains of the colon bacillus. The diminution of effect thus may be selective as the result of the X-ray treatment. Likewise in three instances, treatment of the cultures reduced the activity of the bacteriophage upon them. Again the effect may be selective inasmuch as this same treated suspension when brought into contact with other strains of *B. coli* bacteriophage may still show the usual lysis. The effect, therefore, cannot be ascribed to sterilization of the culture in all instances for they were not killed by this treatment.

4724

Parenteral and Gastro-Intestinal Types of Proteolysis.*

H. C. SOX, H. C. TORBERT AND W. H. MANWARING.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

In a previous note[1] we reported that under the influence of canine leucocytic extract horse proteins undergo an initial pseudo-proliferation, presumably due to immunologically symmetrical proteolysis. A similar apparent multiplication of horse proteins takes place on 14-day incubation with an excess (20:1) of normal canine serum. In both cases the apparent multiplication is followed by a marked flattening and distortion of the precipitin graph[2] which we interpret as an index of horse protein denaturization.[3]

In contrast with this parenteral type of proteolysis, gastro-intestinal proteolysis has thus far given in our hands no suggestion of pseudo-proliferation and subsequent partial denaturization. If 5% horse serum is added to 0.1% commercial trypsin in Ringer's solution, for example, or to 0.1% commercial pepsin in acidulated (n/100) Ringer's solution and the mixture is incubated at 37.5° C. for several hours, precipitin graphs show from the first a rapid and consistent decrease in horse-protein titer, without flattenings or distortions of the precipitin graph. We interpret this as meaning that in gastro-intestinal proteolysis none of the lytic products retain their original horse protein specificity.

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[1] Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 72.

[2] For typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

[3] Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 110.

This study represents selected data from 20 titrations of test-tube lytic products. Each titration was accompanied by parallel tests of from 2 to 4 non-lytic controls.

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* In discussing the above paper, it was suggested by Prof. C. L. A. Schmidt that the term "depolymarization" be substituted for "immunologically symmetrical proteolysis."

4725

Precipitin Variants.

J. L. AZEVEDO AND W. H. MANWARING.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Anti-horse precipitin withdrawn from a rabbit 10 days after injection of the final immunizing dose of horse proteins gives precipitin graphs[1] suggesting a 100% 17th-day retention of intravenously injected horse proteins in the canine circulation, with marked denaturization of these proteins.[2] Parallel tests with precipitin withdrawn 23 days after the final immunizing dose, give graphs suggesting but 1% horse protein retention with no horse protein denaturization.

Our conventional assumption that the specific precipitin is biochemically identical at all stages of sensitization and immunization with the same antigen evidently requires further study.

This report gives the maximum difference observed in 12 parallel titrations of parenteral horse protein derivatives with about 20 different rabbit antisera.

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[1] Azevedo, J. L., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

[2] Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 110.

4726

Effect of Sulphur Dioxide on Vitamin A Activity of Cod Liver Oil, Butter, and Alfalfa.

OSMAN HORACE CADY AND JAMES MURRAY LUCK.

From the Department of Chemistry, Stanford University.

The work here presented was conceived by the observations of Bills[1] on the action of various reagents on the antirachitic vitamin, and was carried to completion in 1927. It occurred to us that an extension of Bills' work to vitamin A might be of value in throwing light on the chemical nature of this factor.

Of the reagents examined by Bills, sulphur dioxide was found to be without action on the antirachitic vitamin. Early in our investigation, however, we found the effect of this substance on vitamin A in cod liver oil to be of such a magnitude as to render desirable an examination of vitamin A from other sources.

In doing so we were guided by two principal considerations: (1) whether vitamin A activity is the property of a single chemical individual or that of a specific atomic grouping possessed in common by several different molecules; (2) assuming that vitamin A of the green plant would be as susceptible to destruction by sulphur dioxide as vitamin A of animal origin, it occurred to us that one important aspect of the damage to vegetation by smelter smoke would be the destruction of vitamin A. With these considerations before us we gave primary attention to experimentation with sulphur dioxide. Cod liver oil, butter, and extracts of alfalfa were used.

Cod liver oil was treated by bubbling with the purified gas at temperatures of 20° to 100° for periods of 15 minutes to 2 hours. The excess of sulphur dioxide remaining at the end of the experimental time was removed by exposure *in vacuo*.

Butter was melted at 60°, freed of casein by filtration, and treated with sulphur dioxide at 60° for 1 to 24 hours.

Alfalfa "leaves and blossoms" of high vitamin A activity were exhaustively extracted at 35° with many portions of alcohol for a total period of 35 hours. The combined extracts were concentrated on a steam bath and divided into 2 portions. One portion was reserved for the control diet, the other was treated with sulphur dioxide by bubbling at 60° for 1 hour. The cod liver oil, butter, and alfalfa extract, treated and untreated, were added to dextrin and incorporated in the diet in various known proportions. 119 animals were used in the experiments on cod liver oil, 54 on butter, and 103 on alfalfa and spinach. In testing for vitamin A the growth method of Drummond and Coward[3] and the curative method of Steenbock and Coward[4] were employed. The results may be summarized as follows:

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[1] Bills, C. E., *J. Biol. Chem.*, 1925, lxiv, 1.

[2] Steenbock, H., *Science*, 1923, lviii, 449.

In cod liver oil, treatment with sulphur dioxide for as short a time as 15 minutes at room temperature was sufficient to cause a complete loss of the growth-promoting, anti-xerophthalmic activity. The destruction was complete as indicated by feeding the treated oil over a wide range (0.25 to 5% of the whole ration). Under fairly similar conditions (1 hour at 60°) the destruction of vitamin A in alfalfa extracts was inappreciable. Butter occupied a somewhat similar position. Though treated for 2 hours at 60° and fed at a level of 5%, the extract continued to show marked vitamin A activity. Even after 22 hours of treatment much of the activity remained. When fed at the lower level of 2%, partial destruction of the vitamin became evident.

In conclusion, the vitamin A activity of cod liver oil is to be regarded as the property of a substance or substances not contained in significant quantities in butter and extracts of alfalfa, or else vitamin A of plant origin is associated with a protective substance not found in cod liver oil. These alternative explanations will be discussed in the final report with the assistance of additional experimental data on the inactivation of vitamin A.

We wish to acknowledge the interest of Professor R. E. Swain, by whom the problem was suggested.

4727

Effect of Adrenalin and Asphyxia on Blood Sugar of Amytalized Dogs.

GEORGE GIRAGOSSINTZ AND J. M. D. OLMSTED.

From the Division of Physiology, University of California, Berkeley.

Recent work has thrown doubt on the presumption that use of amytal (isoamyl ethyl barbituric acid) as an anesthetic is without interference to blood sugar regulation.[1] In spite of the finding of

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[3] Drummond, J. C., and Coward, K. H., *Biochem. J.*, 1920, xiv, 668.

[4] Steenbock, H., and Coward, K. H., *J. Biol. Chem.*, 1927, lxxii, 765.

[1] Olmsted and Giragossintz, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 103.

Page[2] that amytalized rabbits and dogs show normal rise in blood sugar upon splanchnic stimulation, it seemed advisable to determine whether other methods of provoking hyperglycemia would produce normal results. The dosage of amytal employed in our experiments was 50-60 mgm. per kilo intraperitoneally.

The first method tried was intravenous injection of adrenalin. To test our sample of adrenalin 0.5 cc. of a 1 :1000 solution was in-

TABLE I.
Blood Sugar after Asphyxia.

	Before Amytal	After Amytal				After Asphyxia				
		½ hr.	1 hr.	2 hrs.	3 hrs.	5 min.	15 min.	30 min.	45 min.	1 hr.
No. 1 Normal dog	87					152				
No. 2 ” ”	95					182	149	125	111	111
No. 3 Amytalized dog	95	100	100	96	100	102				
No. 4 ” ”	100	100	105	96	85	85				
No. 5 ” ”	125	121	125	120	120	119				
No. 6 ” ”	100					110	107	103		98

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[2] Page, *J. Lab. and Clin. Med.*, 1923, ix, 194.

jected into a 16 kilo normal dog, whose post absorptive blood sugar was 0.080. There was no change in 5 minutes, but 30 minutes later the blood sugar had risen to 0.160. In a 21 kilo amytalized dog 1 cc. of 1 :1000 adrenalin caused a rise of blood sugar from 0.105 to 0.190 within 5 minutes, 30 minutes later it was 0.185. In an 11 kilo amytalized dog 0.5 cc. 1 :1000 adrenalin caused a rise from 0.100 to 0.200 in 5 minutes and 30 minutes later it was 0.192. Injection of adrenalin produces normal hyperglycemia in amytalized dogs.

The second method used was asphyxia. A sheet of rubber dam was wrapped about the dog's muzzle and its air supply entirely cut off for exactly 2 minutes. The dogs' tongues were found to be cyanosed when the bandage was removed. The results are given in the accompanying table.

In none of the amytalized dogs did asphyxia produce the customary rise in blood sugar. Dog 6 was so severely asphyxiated that 5 minutes artificial respiration was necessary before it would breathe on its own. Even this degree of asphyxia produced no significant change in blood sugar. It was noted that the rapid breathing which normally follows asphyxia was absent in the amytalized dogs.

Western New York Section.

Clifton Springs Sanitarium, December 14, 1929.

4728

Ammonium Carbamate as the Intermediate Product in the Action of Urease on Urea.

JAMES B. SUMNER AND DAVID B. HAND.

From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca.

A method for quantitative estimation of ammonia nitrogen and carbamic acid nitrogen, in a solution containing ammonium salts, urea, and carbamates, has been developed. The method depends on the fact that ammonium carbamate is stable in Nessler solution so that one-half of the nitrogen in the ammonium carbamate does not affect the color of the Nessler solution. However, if the solution is acidified before Nesslerizing, the carbamic acid is almost instantly decomposed and Nesslerization gives the total of ammonia and carbamate nitrogen present. In other particulars analyses are made according to the well-known colorimetric method of determining ammonia. Each solution to be analyzed was Nesslerized under these 2 different conditions. The difference between the two values for nitrogen was equal to the carbamic acid nitrogen. Twice this figure was equal to the ammonium carbamate nitrogen. The method was shown to be quantitative within the limits of accuracy of reading a colorimeter.

A number of experiments have been carried out in which the enzyme was allowed to act on 100 cc. ice-cold 1% urea. The action was stopped after 5 to 7 minutes by adding a trace of potassio-mercuric iodide. During this time about 1 mg. per cc. of total combined nitrogen had been liberated from the urea. This concentration was found to be most convenient for analysis. After poisoning the enzyme, analyses were made immediately for ammonium carbamate and ammonium carbonate and then at intervals until the carbamate had fallen almost to zero. Figure 1 shows the curve for

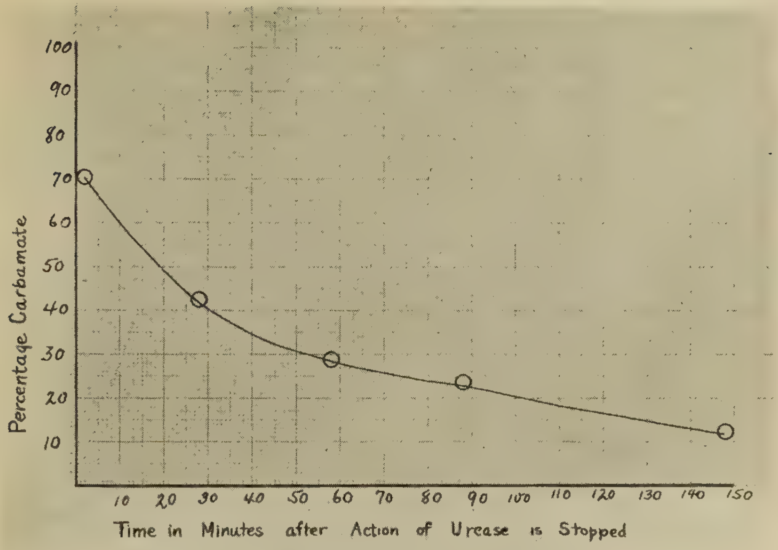

FIG. 1- Decrease in Percentage Carbamate with Time.

FIG. 1.

percentage ammonium carbamate nitrogen of the total nitrogen when plotted against time after the enzyme had been stopped.

The percentage of carbamate diminished very rapidly but at a measurable rate. Our method of analysis was not accurate enough to determine the point of equilibrium between the carbonate and the carbamate. An almost identical curve was obtained when 261 mg. of pure solid ammonium carbamate was added to ice-cold water and the percentages of carbamate nitrogen plotted against time of analysis. When the enzyme was allowed to act in acetate and phosphate buffers no carbamate could be detected. In sodium bicarbonate, carbon dioxide buffer at pH 9, the carbamate formed was detectable but disappeared very rapidly. It is known that ammonium carbamate decomposes quite slowly in presence of excess carbon dioxide. We accordingly allowed urease to act on urea in a solution kept acid with CO_2. 100 cc. of 0.2% urea were cooled with ice and CO_2 bubbled slowly through. Crystalline urease was added and allowed to act for 15 minutes and the reaction stopped with mercury. The first analysis was made 16 minutes after adding the enzyme and 84.5% of the nitrogen was in the form of ammonium carbamate.

These experiments we take to prove that an intermediate compound is formed by the action of urease which contains only half its nitrogen in the free ammonia form. The compound is identified as ammonium carbamate because its rate of hydrolysis is comparable to ammonium carbamate and is affected in the same way by acid, alkali, ammonia and carbon dioxide. The proof rests on the fact that no cyanate is formed by the enzyme.

We conclude that carbamate is formed in large amounts during the action of urease on urea in alkaline solution and that after the urease has been inactivated the carbamate decomposes to carbonate at a measurable rate. Our experiments indicate that all of the ammonia liberated from the urea passes through the ammonium carbamate stage. We hope to make further analyses investigating this point. There is no evidence of any intermediate product other than ammonium carbamate.

4729

Phospholipid Content and Physiological Activity of Tissues.

W. R. BLOOR.

From the University of Rochester, School of Medicine and Dentistry, Rochester.

In tissues in differing states of physiological activity the phospholipid percentage was greater when the tissue was more active. In the corpus luteum of the pig the phospholipid content was about 3 times as great during its active state in ovulation and pregnancy as in the resting state. Cholesterol was not greatly different. Malignant tumors had about 3 times the content of phospholipid and twice the content of cholesterol as benign tumors. Mammary glands (rabbit) at the end of pregnancy had twice the phospholipid content of the resting gland, cholesterol remaining the same.

4730

The Polynuclear Count in the Rat, with Special Reference to Vaginal Smears.

J. FRANKLIN YEAGER AND H. O. HATERIUS. (Introduced by Eric Ponder.)

From New York University, Washington Square College.

The presence of polynuclear leucocytes in vaginal smears of the rat has been noted by Long and Evans,[1] who observed the presence of such cells during certain phases of the oestrous cycle, no quantitative measurements being made. The present report deals with polynuclear counts made from blood and from vaginal smears taken simultaneously from the same animal. All data were obtained from healthy rats kept under ordinary laboratory conditions.

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[1] Long, J. A., and Evans, H. M., *Univ. of Calif. Pub.*, 1922, vi.

TABLE I.

Polynuclear count of blood and of vaginal smears.

Stage of cycle	Polynuclear counts											
	Blood						Vaginal					
	I	II	III	IV	V	Weighted means	I	II	III	IV	V	Weighted means
Oestrus	88	12	0	0	0	1.12						
Metoestrus	93	5	2	0	0	1.09						
Dioestrus	86	12	2	0	0	1.16	48	27	13	8	4	1.93
Dioestrus	84	15	1	0	0	1.17	62	28	7	1	2	1.53

Blood smears were prepared in the usual manner. The smears, upon drying, were fixed with methyl alcohol for one minute and were stained subsequently with Wright's blood stain. A few smears were stained with "Fadicit", only Solution I being used. Either stain permits an easy identification of the polymorphonuclear neutrophiles and counts may be made without difficulty.

Vaginal smears, obtained by means of a wire loop and clean cotton, were stained with Delafield's hemotoxylin. In making a polynuclear count, cells showing fragmentation or obvious distortion were omitted, the count including only cells possessing a distinct cytoplasmic outline.

It will be noted from Table I, a representative cycle, that the blood count is decidedly left-handed as compared to that of normal man, and that the vaginal smear count, while also decidedly left-handed as compared to man, is also somewhat right-handed when compared to the animal's own blood count. The average weighted mean of a group of 35 blood polynuclear counts is 1.09, with a standard error of ±0.0016, while the average weighted mean of a group of 20 vaginal polynuclear counts is 1.69, with a standard error of ±0.039.

The polynuclear count of the rat is similar to the polynuclear counts obtained by Simpson[2, 3] from the cow, the sheep and the horse. Each of these animals presents the interesting condition, therefore, of having most of the polynuclear leucocytes of the blood fall into the first 2 classes of the polynuclear count.

4731

Effects of High Voltage Cathode Rays upon Germinal Epithelium of the Rat.*

VICTOR C. JACOBSEN.

From the Department of Pathology, Albany Medical College, Albany, N. Y.

A study was made of the effects of high voltage cathode rays on the spermiogenic epithelium of the male adult white rat. The animals were about 5 months old, apparently healthy and virile. The shaved scrotal area was exposed in front of the anodal window of the Coolidge cathode ray tube to a current of one milliampere, at voltages of 200,000 and 250,000, about equally divided as to number of animals. All raying of more than one second duration was given in one second periods with about one-half second between each.

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[2] Simpson, Ethel D., *Quar. J. Exp. Physiol.*, 1929, xix, 309.

[3] Simpson, Ethel D., *Quar. J. Exp. Physiol.*, 1929, xix, 317.

* This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

Tissues studied one to 50 days after raying.

Protocol of the experiments:

No. of rats	Time of exposure	Voltage
3	0.5 sec.	200,000
6	1.0 "	" "
8	5.0 "	" "
4	10.0 "	250,000
4	15.0 "	" "
3	20.0 "	" "
3	30.0 "	" "
6	normal controls	

The changes in the scrotal skin were similar to those in the abdominal skin as described previously by Jacobsen and Waddell,[1] and consisted chiefly of hyaline fusion of the collagen of the corium, acute necrosis of epidermal and hair follicle epithelium, with vascular changes as a much later occurrence.

Definite lesions were produced in the seminiferous tubules in a zone about 0.6 mm. in depth in the animals exposed for from 20 to 30 seconds, with proportionately shallower penetration in the animals exposed for shorter times. Pyknosis, fragmentation and karyolysis of nuclei, and depression of mitosis were seen in spermatogonia, spermatocytes, and spermatids, the Sertoli cells being least affected. The interstitial cells showed insignificant changes. The *tela subcutanea* below the upper 0.2 mm. of the skin and the *tunica vaginalis testis* showed no evidence of injury, indicating very clearly the much greater sensitivity of germinal epithelium to this form of radiation.

Alterations in the Golgi apparatus of the germinal cells were also observed, fragmentation, perinuclear arrangements and fusion with acrosomic material, changes corresponding with findings in the roentgen-rayed testis of *cavia cobaya* as reported by Gatenby and Wigoder.[2]

These observations, resembling as they do those made on roentgen-rayed testis, are offered as further biological evidence that the effects of roentgen rays are not due primarily to roentgen rays but to the electrons or cathode rays set free in the tissues.

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[1] Jacobsen, V. C., and Waddell, K. C., *Arch. Path.*, 1928, v, 195.

[2] Gatenby, J. B., and Wigoder, S., *Proc. Roy. Soc.*, 1928, civ, 351.

4732

A Further Report on the Effect of Thyroparathyroidectomy on the Action of Irradiated Ergosterol.

A. F. HESS, M. WEINSTOCK AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a recent note[1] we reported that calcium in the blood could be raised to abnormal levels in dogs and in monkeys by feeding large amounts of irradiated ergosterol, but that hypercalcemia could not be induced by this means after the parathyroids and thyroids had been extirpated. In these experiments, the amount of ergosterol which was given was usually about 40 times that which is prescribed for infants of equivalent weight.

In the course of subsequent experiments of this kind, we have found that if still larger amounts of irradiated ergosterol are given, it is possible to induce hypercalcemia, after the thyroid and parathyroid glands have been excised. For this series of tests, 100 to 400 times the therapeutic dose has been given; in some instances as much as 800 times this dosage has been fed. Under such conditions, the calcium may rise from 6 or 7 mg. to 17 or even 20 mg. per 100 cc. of blood. That the parathyroids had been completely removed from the body, was shown furthermore by the fact that the inorganic phosphorus in the urine became markedly decreased or even was entirely absent following the operation.

As stated previously[2] we are of the opinion that the source of the marked increase in serum calcium induced by excessive amounts of irradiated ergosterol is the tissues, more particularly the bones, which are the great storehouses of calcium in the body. Subsequent investigations have confirmed this point of view. If dogs are fed, for a period of weeks or even months, on a diet which is almost free of calcium, being composed of cracker meal, dried meat and mazola (corn) oil, the calcium of the blood can be raised to abnormal levels by feeding excessive amounts of irradiated ergosterol, in spite of the deficiency of calcium in the ration. Furthermore, metabolism tests on dogs treated in this way show that there is a lack of retention of calcium, either a definitely diminished or a negative balance. It may be added that in rats which were given excessive amounts of irradiated ergosterol, the ash of the bones was lower than in those of the control animals which had not received this addition.

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[1] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 55.

[2] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 199.

It is our opinion, therefore, that very large amounts of activated ergosterol are able to withdraw calcium from the bones and that, if the dose is sufficiently large, they are able to accomplish this after the parathyroid glands have been excised. It should be emphasized, however, that this conclusion does not hold true for therapeutic doses of this drug, for it has been satisfactorily shown[3] that small amounts of irradiated ergosterol bring about a retention of calcium, as is true of cod liver oil and of direct ultra-violet irradiation.

4733

The "S" and "R" Forms of Hemophilus Influenzae.

MARGARET PITTMAN. (Introduced by Rufus Cole.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

During the course of an experiment with a culture of *Hemophilus influenzae,* transplants were made on a plate of Levinthal's solid transparent medium. It was observed that the culture contained 2 types of colonies. Well defined small transparent colonies resembling the textbook description of the typical Pfeiffer bacillus colony were found but in addition there were larger, slightly opaque iridescent mucoid smooth colonies. Transplants were made from the transparent colonies on Levinthal agar and all the colonies were found to be identical with the mother colony. The same was true of transplants from the opaque iridescent colonies. It was thus possible to separate 2 cultures each of which produced typical colonies. Since the largest transparent colonies were found to have a rough surface the strain producing these colonies has been called an "R" strain; and since the iridescent colonies from the other culture had a smooth surface this strain has been called an "S" strain.

This culture and 2 others from which "S" and "R" forms were subsequently isolated had been seeded many times on blood or chocolate agar and the variation in the colonies had not been noticed. Yet the difference in the colony appearance was marked when transplants were made on the transparent agar plates. Very striking was the difference when the plate cultures were held before a 100 watt electric light bulb or in the sunshine.

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[3] Shohl, A. F., Bennett, H. B., and Weed, K. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 551.

The morphology of the bacteria from the 2 strains has also been found to be dissimilar and characteristic for each type. This is best noticed if the stained preparation is made from a solid medium culture which is not older than 24 hours. The bacteria from the "S" culture appear as small coccobacilli that vary little in size. When preparations are made with special capsule stains there is definite indication of capsular substance, although the present methods of staining have not demonstrated a capsule that is as definite as is the capsule on the Pneumococcus. The bacilli from the "R" culture vary in length from coccobacilli to very long thread forms. The rougher the colony, the more anomalous is the morphology of the organisms; and with capsular stains we have failed to demonstrate the presence of any capsular substance.

An "S" and its corresponding "R" strain seem to be identical in their hemoglobin requirements and in their ability to form indol, to reduce nitrates to nitrites and to ferment the same sugars. To be added to these biological tests is a new one which is very interesting and probably significant. It is that both "S" and "R" strains of influenza bacilli are soluble in bile and desoxycholic acid.

At present we have 4 "S" strains. Three of these, mixed with "R" strains, were found among cultures which had been carried from 5 to 9 months. One of these cultures was from a case of meningitis, another was from the blood of a patient with pneumonia, and the third was isolated from the sputum of a case of broncho-pneumonia. Whether all the bacteria of these cultures were of the "S" form when originally obtained from the patient cannot be stated. But the fourth "S" strain, which was recently isolated from a case of broncho-pneumonia, was not associated with "R" forms.

It has been possible to change with great readiness all "S" strains into "R" strains by making daily transfers in 10% homologous anti-S serum broth. On the other hand, an "R" strain which had been converted a short time before from an "S" strain, has been reverted to its original "S" form by transferring it in anti-R serum broth.

The filtrate from a 20 hour old broth culture of the "S" strain contains a substance which is precipitable in homologous anti-S rabbit serum. Anti-R serum does not precipitate the S filtrate. And the filtrate from the "R" culture is precipitated neither by the anti-S nor by the anti-R serum. With the filtrates of the "S" strains so far isolated two distinct serological types have been observed. These results suggest that the "S" organisms produce a soluble specific substance which may be analogous to the soluble specific substance produced by Pneumococcus.

4734

Filterability and Mechanisms of Excretion of Certain Dyes by the Frog's Kidney.

JEAN OLIVER and ESHREF SHEVKY.

From the Departments of Pathology of Stanford University Medical School and the Long Island College Hospital Medical School.

This report concerns a correlation of the filterability of certain dyes with the manner of their excretion by the kidney as tested by the method of perfusion. The details of the technique of this method may be found in previous publications[1] and the results of our present experiments briefly summarized as follows:

Filtration of a series of dyes through collodion membranes gave the following average values of filterability. Phenol red, 100%; indigo carmine, 90%; toluidin blue, 60%; and neutral red, 35%. Trypan blue was unfilterable as such, but the pink component of the dye passed through the filter in small amounts, and brilliant red was entirely unfilterable.

By perfusion of either the glomerular or the tubular circulation of frogs with these dyes, it was found that phenol red and indigo carmine were eliminated chiefly through the glomeruli. Toluidin blue and neutral red on the other hand were excreted principally by the tubules. Trypan blue was excreted in the relatively short period that our experiments continued only in traces, and these traces passed through the glomeruli most readily and showed the same separation of the components of the dye as was observed in the filtration process, for the urine was pink in color. Brilliant red did not pass through either tubules or glomeruli.

Anesthesia of the tubules and repression of their function during the course of dye excretion increased the rate of elimination of phenol red and indigo carmine, but decreased the rate of excretion of toluidin blue and neutral red. When the glomeruli were damaged in such a way as to increase their permeability, both components of trypan blue escaped into the urine, which assumed the bluish color of the original dye solution. Damage to the glomeruli also allowed the passage of brilliant red through the glomeruli.

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[1] *J. Exp. Med.*, 1929, l, 15.

These findings are summarized and correlated in Table I.

TABLE I.

Filterability	Dye	Glomerular excretion	Tubular excretion
Easily filterable	Phenol red	Marked	Slight
	Indigo carmine	Marked	Slight
Moderately filterable	Toluidin blue	Moderate	Moderate*
	Neutral red	Slight	Marked
Slightly filterable	Trypan blue	Pink element—trace	Slight
Unfilterable	Brilliant red	None	None

* Some tubular damage from dye.

We interpret these results as indicating that dyes of small particle size pass readily through the glomerular filter whereas those of less filterability require the activity of the tubular epithelium for their elimination. The similar separation of trypan blue into its components by the intact glomerular membrane is further evidence that the processes of glomerular excretion and filtration are analogous.

It was later noted that neutral red in the acid form was excreted at a much greater rate than the alkaline form. Further analysis showed that this increase was due to an increased passage of this dye through the glomerulus, a process which as we have just noted is relatively slight under physiological conditions of acidity.

A new examination of the filterability of neutral red, comparing the alkaline and acid forms showed that the latter was from 3 to 4 times as filterable as the alkaline form. Direct observation of the two forms by ultramicroscopy proved that this increase in filterability was due to a decrease in the size of the dye particles.

It would seem, therefore, that one very important factor in the determination of the mechanism of the elimination of a dye, whether glomerular or tubular, is the size of the particles into which the substance is dispersed.

4735

Gastric Hypersecretion Following Parturition in a Dog.

FRANKLIN HOLLANDER. (Introduced by I. S. Kleiner.)

From the Department of Physiology, New York Homeopathic Medical College and the Biological Laboratory, Cold Spring Harbor, L. I.

It has been shown previously[1, 2] that, in the collection of gastric juice from dogs with fundus pouches of the Pavlov type, the combined acidity becomes negligibly small if the secretion be collected in such a way as to minimize its content of mucus or serous transudate. With this elimination of combined acid, the free acidity, as determined in 4 dogs, gave an average value of 0.157 N ± 0.003 N. During the past summer, several more such fundus-pouch animals were prepared, one of them a pregnant dog of 10 kilos body weight. Five weeks after the operation, this animal dropped a litter of 4 healthy pups which she suckled as long as she was permitted to.

For the 2 weeks immediately preceding parturition, the total volume of juice collected from her pouch never exceeded 15 cc. per day—this amount being secreted wholly within 6 to 8 hours after feeding. Beginning with the day of parturition, however, the flow increased very markedly in amount and duration. In fact, except during certain periods of experimentation, the secretion was practically continuous throughout the 24 hours between single daily feedings. For the first 3 weeks of lactation, the average daily volume of juice collected was 150 cc. A number of times the pouch was so full that the pressure forced evacuation with consequent loss of contents. The increase in daily output, therefore, was well over tenfold. During the sixth week, however, the second week after the pups had been completely weaned, the average daily output had fallen to about 30 cc. and was decreasing further at the time of the accidental death of the dog during the seventh week.

Titration of 66 samples of this hypersecretion juice gave an average value of 0.157 N ± 0.007 N for total acidity—titrating with phenol red to pH 7.1. Combined acidity, measured by the difference between free and total, was negligibly small. The agreement between these and the previously observed normal values is such as to validate the inference already made by other workers from clinical observations, that the acidity of the secretion from the parietal cells is inde-

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[1] Hollander, F., *J. Biol. Chem.*, 1927, lxxiv, xxiii.

[2] Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 486.

pendent of the daily output of this product, *i. e.*, of the amount of work these cells do.

Further investigation of this phenomenon is already under way.

4736

Agglutination of "Normal" Germ Cells.

A. J. GOLDFORB.

From the Department of Biology, College of the City of New York.

It is widely believed that freshly shed germ cells are relatively constant. In previous studies[1, 2, 3, 4] an extremely wide variation in many characters and characteristics was noted. In the present study agglutination of sperm by egg water, which agglutination lends itself to fairly exact quantitative determination, was studied under very precise experimental conditions.

The sea urchin *(Arbacia punctulata)* was used. These were freshly collected, opened immediately upon arrival at the laboratory, the "best" egg lots selected and immediately tested for the duration of agglutination. Duplicate tests and tests of aliquot divisions of the eggs gave a difference of 0 to 4 seconds, averaging 1 second or 4.5%. This was considered the experimental error.

Thirty series of 3 to 4 females each were studied. When eggs from each female in a series were tested separately, under strictly comparable conditions (including the same sperm suspension), the agglutination time varied very extensively, namely, from 2 to 55 seconds, or 9 to 1300%, with an average of 12 seconds or 142%.

Eggs that gave high agglutination values with sperm from one individual gave consistently high, though not the same values, with sperm from other individuals. Similarly eggs that gave intermediate or low values with one male, gave intermediate or low values with other males.

This wide variation and increase in agglutination time parallels corresponding wide changes in size, color, shape of eggs, loss of jelly, membrane formation, cleavage, etc.[1, 2, 3, 4] The degree of change in any one of these traits measures the extent of overripening of the eggs prior to shedding.

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[1] Goldforb, A. J., *Proc. Nat. Acad. Sci.*, 1917, iii.

[2] Goldforb, A. J., *Carnegie Inst. Publication* 251, 1917.

[3] Goldforb, A. J., *Biol. Bull.*, 1918, xxxiv.

[4] Goldforb, A. J., *Biol. Bull.*, 1918, xxxv.

It was also noted that severe storms that delay natural shedding, also higher temperature of the sea water, give rise to overripening of the eggs within the body of the sea urchin, with all the symptoms above enumerated.

Not only were the eggs of different individuals in widely different stages of overripeness, even when freshly shed, but sperm from different males freshly collected, immediately tested, in the same concentration of sperm suspension with the same egg water, gave widely different agglutination values, namely, 11 to 3300%. This wide range in agglutinability of different sperms is interpreted to indicate corresponding differences in overripeness prior to shedding.

Sperm which gave high agglutination values with one female, gave high, though not the same, values with other females, and *vice versa,* sperm which gave intermediate or low values with one female gave correspondingly intermediate or low values with other females.

This extremely large variation in freshly shed germ cells may in very small part be due to genetic differences. But the major differences appear to be due to corresponding degrees of overripeness prior to shedding.

"Normal", *i. e.,* chronologically freshly shed germ cells may be physiologically fresh eggs, or they may be in nearly all stages of physiological deterioration or overripeness. As the subsequent behavior of the fertilized eggs depends upon the physiologic condition of the germ cells at the time of fertilization, it is therefore necessary, in experimental work, to determine by the agglutination or other tests their exact physiologic condition prior to experimentation.

4737

Agglutination Changes in Ageing Germ Cells.

A. J. GOLDFORB.

From the Department of Biology, College of the City of New York.

A. When freshly shed eggs of Arbacia were physiologically ripe (not overripe) and then aged in sea water at 20° C. there occurred a profound series of changes in agglutination. Three phases may be readily distinguished. In the first phase there was a progressive and marked *increase* in agglutination, reaching a maximum of about 200% in 3 to 5 hours. This increase is due to a corresponding increase in liberation of agglutinin. In the second phase there was a progressive decrease in agglutination, namely, from about 5 hours to 24 hours. This decrease is associated with a decrease in agglutinin. In the third phase there was continued decrease in agglutination, due to liberation of anti-agglutinin.

When eggs were overripe at the time of shedding, corresponding portions of the first or even second phase did not occur. These phases took place prior to shedding.

Overripening gave rise to the same kind and the same intensity of changes whether within or without the body.

B. When freshly shed sperm from different freshly collected males were aged at 20° C. and tested at each age under strictly comparable conditions, by either the same egg water or by freshly shed ripe eggs, agglutination was widely different. There were 3 phases. In the first there was practically no change, *i. e.,* from 0 to about 3 hours. In the second, agglutination *increased* progressively and markedly, from the third to the twenty-fourth hour. The average increase was 120%. In the third phase there was a rapid and progressive decrease in agglutination.

When the freshly shed sperm were overripe, or were precociously overripened by high temperature, corresponding portions of the first or second phase were not evidenced.

C. When the freshly shed eggs and sperm were not overripe, and the same germ cells tested at successive ages, under strictly comparable conditions, there occurred the same cyclical *increase,* then decrease in agglutination. The increase, however, was greater than when eggs alone or sperm alone were aged.

When the freshly shed eggs or sperm or both were overripe changes like those above mentioned occurred, namely, absence of corresponding portions of the first or second phases in the agglutination cycle.

D. Eggs undergo with age a cyclical change in agglutinin. Sperm undergo a corresponding cyclical change. The two cycles are not synchronous. The agglutination at any time depends upon the physiologic condition of the eggs and of the sperm at that time, and is the sum of the effects produced by the eggs and the sperm.

The agglutination phenomenon is conditioned by 4 major factors, viz., (1) degree of overripeness of eggs and of sperm when shed. (2) time or age. (3) condition of ageing, temperature, H ion concentration, etc. (4) different rates of change in ageing eggs, and sperm.

E. Sperm are not a fixed entity reacting in the same way with same intensity to a given dose of agglutinin. Sperm is a varying entity either secreting, with age, varying amounts of a substance or substances which activate agglutination, or, sperm undergo a cyclical change or changes as a result of which they become increasingly reactive to a given dose of agglutinin.

4738

The Factors that Modify Agglutination of Ageing Sperm.

A. J. GOLDFORB.

From the Department of Biology, College of the City of New York.

The previous studies demonstrated that ageing "dry" or concentrated sperm gave rise to a marked increase, then decrease in agglutination, provided, however, the sperm were not overripe when shed. In the present study, the sperm were overripened in a standard 1% suspension at 22° C. and tested at each age by samples of the same egg water. There was the same, but quicker, cyclical change. The first phase began in 5 to 20 minutes after the initial test. The second phase ended in 15 to 125 minutes. The third phase ended in 260 minutes. The increase in agglutination was from 77 to 328%.

The greater the overripeness of the dry sperm when shed, the earlier the maximum values, and the sooner was the cycle ended. When sperm was not overripe when shed, subsequent ageing gave rise to very little or no increased phase, followed by a decreasing phase.

The experiments were so devised as to eliminate such factors as a change in agglutinin, change in jelly content, change in temperature, or H ion concentration of the medium.

To determine whether the increase in agglutination was due to a substance or substances liberated by the sperm, increasing concentrations of sperm were tested with the same egg water solutions. The concentrations of sperm ranged from 1% to 25%. More concentrated suspensions could not be used, on account of the difficulty in distinguishing the agglutinated clusters in the thick medium. Sufficient time was allowed for the substance if present to be liberated. There was no change in agglutination values with the marked increase in concentration of sperm whether ripe or overripe.

The CO_2 liberated by the eggs and the active sperm plays a large rôle in decreasing the activity of the sperm, in clumping the sperm into aggregations, but plays no rôle in increasing the agglutination of ageing sperm.

This increase with overripening of either dry sperm or sperm in suspension is therefore held to be due not to a substance liberated by sperm but to a physiologic change in ageing sperm (as in the case of eggs). The physiologic changes include a change in metabolism, in permeability of the sperm wall, in viscosity of the protoplasm, and in a changed reactivity to a given dose of agglutinin. This cyclical change in reactivity is paralleled in the eggs by a cyclical liberation of agglutinin.

It is this cyclical physiologic change in eggs and in sperm which is held responsible for the initial vitalizing phase, the later devitalizing and senescent phases.

The results and interpretation with ageing sperm are in harmony with those obtained in agglutination of bacteria.

4739

Effects of Oxygen Therapy on Patients with Congestive Heart Failure.

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The effects of living for 2 to 5 weeks in an atmosphere containing 40 to 50% oxygen were studied in 5 patients with cardiac insufficiency. The Barach oxygen chamber, with constant temperature and humidity regulation, was used. Studies were made (a) with the patients in the ward, both before and after residence in the oxygen chamber; (b) with the patients in the oxygen chamber with normal (21%) atmospheric oxygen content; and (c) with the patients in the oxygen chamber with 40 to 50% of oxygen in the atmosphere. All other procedures, such as diet, fluid intake and the dosage of drugs, were kept sufficiently constant so that they did not modify the results. The usual clinical observations were recorded and in addition, measurements were made of the basal metabolic rate, pulmonary ventilation, vital capacity, arterial oxygen content, arterial CO_2 content, and the CO_2 dissociation curves of the arterial blood. From the latter were calculated the arterial oxygen saturation, arterial CO_2 tension and the arterial serum pH.

In one patient with mitral stenosis of long standing, great cardiac enlargement and marked chronic passive congestion but no peripheral edema, very little change occurred after a week in 45% oxygen. There was only a slight rise in the level of the CO_2 curve while he was in the chamber. No clinical improvement was noted.

Two patients with chronic valvular heart disease, marked decompensation and edema, together with fever and other evidences of active rheumatic infection, showed moderate subjective improvement. In these individuals, residence for 2 weeks in 45% oxygen was associated with a considerable rise in both the arterial CO_2 content and the level of the CO_2 curve. In one case, there was a rise in arterial oxygen saturation from 84 to 93%. (No measurement was made in the other case.)

During the first period in the chamber, when the inspired oxygen percentage was high, there was no marked change in the water balance. Later, the patient who was more seriously ill was removed from the oxygen room to the ward and there occurred rapid and extreme decompensation. The pulse became thready, the blood pressure fell from 156/80 to 90/?, and cyanosis was marked. Coincidently, the oxygen saturation fell from 93 to 84%, the arterial CO_2 from 41.7 to 26.4 volumes %, and the arterial pH changed from 7.38 to 7.32. There was complete suppression of urine throughout the 20 hours that she remained in the ward, during which time 2 catheterizations were done, without result. She was then transferred back to the chamber into an atmosphere containing 45% oxygen. There was prompt relief of symptoms. The blood pressure rose to 120/75, and 2 hours later she began to void. After 2 days, analysis of the arterial blood showed the oxygen saturation to be 98%, the CO_2 content 49.5 volumes %, and the serum pH 7.45.

Of particular interest were 2 cases of non-rheumatic heart disease with advanced myocardial failure. One of these had arteriosclerosis and extensive fibrosis of the myocardium; the other showed a large aortic aneurysm, partly intrapericardial, with aortic insufficiency, probably of luetic origin. Both had dyspnea, orthopnea and marked edema. Each patient, after a preliminary period of study in the ward, was put into the oxygen chamber, with 45% oxygen, for 18 days. At the end of this time, the oxygen in the chamber was reduced to 20% and maintained at this level for 6 days. The oxygen was then again increased to 45% and was kept at this concentration for several days more. The percentage was finally gradually reduced and the patient was transferred back to the ward. There was striking relief of dyspnea, orthopnea and cyanosis, as well as a tendency toward a lower temperature, during the periods when the patients were inspiring a high percentage of oxygen.

The blood changes were similar to those observed in the preceding group, namely, a rise in the arterial CO_2 and in the CO_2 curve level, a rise in arterial oxygen saturation, decrease in pulmonary ventilation and in basal metabolic rate and, in one case, an increase in the vital capacity. The arterial serum pH was not altered.

The striking features of these 2 cases were the occurrence of diuresis and the consequent disappearance of edema while in an atmosphere rich in oxygen. In both cases, a return to ordinary room air was followed by diminution in urinary output and return of edema. A second period of residence in 45% oxygen again resulted in diuresis and disappearance of edema. The direct relationship between the oxygen percentage in the atmosphere and the water balance, as measured by fluid intake and output, seemed clearly established. No such constant and parallel relation was apparent between diuresis and the changes in other measurements, such as vital capacity, metabolic rate, pulmonary ventilation, CO_2 level and hydrogen ion concentration. Probably there was a fairly close association between the amount of urinary output and the degree of arterial oxygen unsaturation, but the data were not sufficiently complete to determine this point. The temperature and humidity of the atmosphere were not deciding factors, because the same sequence of events occurred whether the patient was transferred from the oxygen chamber to the ward, or was kept in the oxygen chamber while the oxygen in its atmosphere was changed from 45 to 21%.

Electrocardiograms were taken at frequent intervals on all 5 patients. No changes were observed in the form of the complexes or in conduction which could be ascribed to variations in the oxygen content of the inspired air.

Citations to literature will be given in the complete report.

4740

A New Type of Microelectrode.

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Several types of microelectrodes have already been devised by different investigators.[1] In all these electrodes the conducting medium, whether of metal or of KCl-agar, is enclosed in a micropipette of glass or quartz. It is possible to make electrodes of similar fineness from metallic wires, but they are too flexible for use.

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[1] Bose, J. C., *Trans. Bose Institute*, 1919, ii; Taylor, C. V., PROC. SOC. EXP.

In the new type of microelectrodes described here, the conducting medium is a continuous film of silver, gold or platinum for stimulating electrodes and silver-silver chloride for non-polarizable electrodes, on the outer surface of glass or quartz micro-needles. The advantages are: (1) they can be easily made and in large numbers at one time, (2) many pure metals can be used, (3) electric contacts of any geometrical shape and fineness can be easily made, (4) the non-polarizable electrode can be made sterile, and (5) their electric resistance is low.

The best way to deposit films of metal on glass and quartz is by spattering, *i. e.,* by placing them in the dark space of high voltage vacuum discharge. But for the deposit of silver films on glass needles, Brashear's[2] method of silvering glass has been found very satisfactory; for bright platinum films Meillere's method[3] is used.

The desired number of micro-needles is drawn in the way described in detail by Chambers.[4] The shank ends of the needles are warmed in a nicroflame and embedded vertically up in a block of paraffin and are then cleaned by immersing the needles vertically down in a beaker of cleaning fluid. After a few hours in the cleaning fluid the needles are thoroughly washed in running water and then kept immersed in a large beaker of distilled water till they are to be placed in the silvering solution. The silvered needles are washed thoroughly and dried in a hot oven.

Electric contacts with these silvered needles are made by inserting them in the electrode holder (Fig. 1). Woods metal previously placed in the tapering end of the holder is melted by gentle heat and

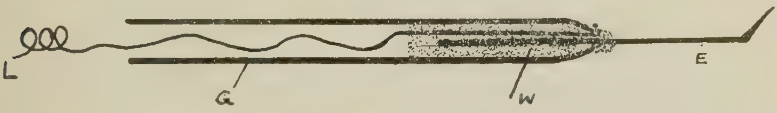

FIG. 1.

Electrode Holder. G, glass tube 4 mm. diameter. W, Woods metal. E, microelectrode. L, copper wire lead.

BIOL. AND MED., 1925, xxiii; Ettisch, G., and Peterfi, T., *Pfluger Arch. f. d. ges Physiol.,* 1925, ccxiii; Gelfan, S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii; Taylor, C. V., and Whitaker, D. M., *Protoplasma,* 1927, iii.

[2] *Scientific American Cyclopedia of Formulae,* 1925, 502.

[3] Clark, W. M., "The Determination of Hydrogen Ions," 1928, 283.

[4] Chambers, R., in McClung's "Test Book of Microscopical Technique," Hoeber, 1929.

the needle thrust in. On cooling of the metal a good contact is secured. Any defect in the rigidity at the contact is overcome by pouring minute drops of melted Woods metal with a fine glass pipette. After mounting the coated needles in this way thicker coats of silver or of any other metal can be deposited electrolytically. For non-polarizable electrodes a thicker coat of silver is desirable. The film of silver chloride is deposited electrolytically by making the electrodes the anode in a 5% KCl bath, the cathode being another piece of silver. With 0.5 volts across the terminals, and keeping the current on only for 30 to 40 seconds a good continuous coat of silver chloride film is obtained. The electrodes are washed and kept in distilled water for a few hours before use. Electrodes when broken are easily replaced by fresh ones by warming the Woods metal of the electrode holder.

When necessary the metallic film of the shank can be insulated by applying shellac solution with a fine brush. The insulation of the micro-tip end is done under a microscope. The tip pointed upward is illuminated in the dark field of the microscope and its shank is gradually lowered by rack and pinion into a small cup of shellac solution. By this method it is easy to insulate up to 10μ from the micro-tip. With care and by using a thicker shellac solution, insulation up to within 5μ can be obtained. The resistance of a pair of electrodes with a micro-tip less than 3μ in diameter has been found to be only 35 ohms. We have been using these electrodes for the last 6 months and they are found to be very satisfactory alike for measuring resistances, by the method previously described,[5] and currents in single living cells and for stimulating single nerves and muscle fibers.

4741

Effect of Cathode Rays Upon Certain Bacteria.

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(Introduced by Peyton Rous.)

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The following experiments, which are intended as preliminary steps in a study of some of the effects of cathode rays upon cells, provide data for a statistical analysis of the rate of killing of cultures of *B. coli*, of *Staphylococcus aureus* and of *B. aertryke.*

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[5] Sen, B., *Roy. Soc. Proc.*, 1923, xciv, 216.

The cathode rays have been obtained from a Coolidge type electron tube operated at a voltage of approximately 200 K.V. Small numbers of the organisms under investigation were evenly spread upon the surfaces of agar plates and known areas were exposed for different lengths of time to the electron stream. After incubation, counts were made of the numbers of colonies growing out in these areas and in similar standard areas shielded from radiation. The ratios of the bacterial colonies in these areas are survival ratios.

A picture of the physical consequences of an electron absorption in a bacterium will be provided by remembering that whenever a fast moving electron is absorbed in matter, some of its energy will be emitted as X-rays but the major part will produce a large number of charged ions within a very small volume. In the present experiments this volume is probably less than 0.001 $mm.^3$ and within it an absorbed electron gives rise to upwards of 10,000 ions. It is natural to attribute the destructive action of cathode rays to the chemical and physical changes resulting from this ionic shower.

By estimating both the number of electrons which strike a bacterium in unit time and the absorption coefficient of these electrons in the bacterium, it is possible to analyse the observed survival ratios by the usual methods of probability theory. Such an analysis will show how many electrons may be stopped by a single bacterium before death results. It will also indicate whether absorption must take place in a particular portion of the organism in order to be lethal.

When the motile bacilli of mouse typhoid were spread on agar so sparsely that not more than about 200 cells were in an area of one square inch, the results agreed with the conclusion that one electron-hit is sufficient to kill. This simple result was only obtained, however, when the effects of cell multiplication were eliminated by irradiating quite immediately after seeding the plates. In order to give a similar one-hit-to-kill relation with the less motile colon bacillus, it was necessary to work with fewer bacteria per plate than with the bacillus of mouse typhoid. Even under the most favorable conditions the data from irradiations of *Staphylococcus aureus* required more than one hit. Microscopic examination of the suspension with which the plates were seeded showed that even at these great dilutions, the cocci were still for the most part associated together in clusters.

The detailed results of the experiments outlined above are considered to justify the following conclusions: (1) For *B. coli* and for

B. aertryke not only is one hit by a 200 K.V. electron sufficient to kill, but every electron absorbed is lethal. The same is undoubtedly true of *Staphylococcus aureus.* (2) These data indicate that the only differences that exist in the resistance of these organisms to 200 K.V. electrons are due to their relative sizes. Since the physical phenomena consequent upon the absorption of an electron are, except for the additional charge borne by the electron, identical with those following the absorption of an X-ray quantum of equivalent energy, it is likely that the specific resistances of these cells to 200 K.V. X-rays can also be quantitatively explained by taking account of their relative sizes. Experiments to bear upon this question are being made with X-rays. (3) These results prove that measurements will lead to valid conclusions concerning the mechanism of the action of the radiation only when extraordinary care is taken to eliminate clumping of cells and to prevent multiplication before raying. These requirements have not been met by the experimental procedures commonly followed in X-ray and ultraviolet research upon bacteria.

4742

The Distribution of Ergosterol Administered to Rabbits.

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Rosenheim's[1] recently announced specific color reaction for ergosterol has been applied to the analysis of tissue from rabbits fed on "Vigantol" (irradiated ergosterol in oil).

The reaction as described by Rosenheim consists in the addition of a saturated aqueous solution of trichloracetic acid to a chloroform solution of the sterol. There is every reason to believe this reaction quite specific for ergosterol or structurally related sterols. We have found after testing many solvents that dichloroethylene (low boiling) is a more satisfactory solvent for the reaction than chloroform. Using this solvent, quantitative estimations of the extracted, partially purified sterols may be made.

Ten adult rabbits were fed "Vigantol" with a stomach tube in doses ranging from 250-870 mg. of irradiated ergosterol. Four were used as controls. Practically all the animals showed the calcification phenomena now well described in the literature. The animals were killed and the organs dried in high vacuum, powdered, extracted with ether (dry), treated with saturated alcoholic barium hydroxide to remove soaps, extracted with petrol ether, the petrol ether evaporated and the residue taken up in dichloroethylene (1 cc.) and treated with one-half cc. of saturated aqueous trichloroacetic acid.

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[1] Rosenheim, Otto, *Biochem. J.*, 1929, xxiii, 47.

The results show a most striking accumulation of ergosterol in the adrenals and brain with small amounts in the liver and kidneys. Muscular organs appear to be almost free from this substance.

Judging from the color reaction the bone marrow especially seems to contain fairly large amount of a $\Delta^{1,2}$ sterin, the nature of which we are now investigating.

The results are all consistent but the amount of ergosterol found by the color test was not in proportion to the amount of ergosterol (Vigantol) fed, *e. g.,* the highest dosed animal did not have a great deal more ergosterol in his body. *A priori* one would of course expect this result.

Human arteriosclerotic aortas and adrenals from elderly patients showing arteriosclerosis contain a substance which strongly suggests by its color reactions and spectrogram the presence of ergosterol and its irradiation products whereas aortas from young individuals dying from varied causes seem to contain none or but traces of these materials.

4743

The Relation of Washed Granules From Commercial Vaccine to the Virus.

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The work of MacCallum and Oppenheimer[1] and of Craciun and Oppenheimer[2] offered at least the hope that the "washed granules" prepared from commercial calf vaccine might represent purified vaccinia virus. We have accordingly investigated further the nature of the washed granular material obtained by the method of Craciun and Oppenheimer.[2]

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[1] MacCallum, W. G., and Oppenheimer, E. H., *J. Am. Med. Assn.*, 1922, lxxviii, 410.

[2] Craciun, E. C., and Oppenheimer, E. H., *J. Exp. Med.*, 1926, xliii, 815.

In confirmation of the earlier work we have found that the granular centrifugalization sediments after repeated washings in Ringer's solution were infectious even in high dilution; characteristic lesions of vaccinia resulted from intradermal injection into rabbits; the supernatant fluids were infectious only in low dilution. When

TABLE I.

Inactivation of Washed Vaccinia Granules at Reactions More Acid Than pH = 5.2–5.3.

Time of exposure	McI. 2.8	McI. 3.3	W. 3.5–3.6	W. 4.0–4.1	W. 4.4–4.5	W. 4.8	W. 5.2	W. 5.3	S. 5.3	S. 5.6	S. 5.9	Granules control
3 hours	—		—	—	—	—	—		+	+	+	++
20 hours in ice box	—		—		—		+		+	+		++
2½ hours at room temp.		—	—	—	—	—			+	+	+	++
2½ hours at room temp.		—		—		tr.		+		+	+	

One volume of each suspension of granules in Locke solution was diluted with 3 volumes of buffer; after the interval indicated, 0.1 cc. of the mixtures were injected into a rabbit. Intensities of "takes" indicated by plus signs. The letters above the columns indicate McIlvaine's citrate, Walpole's acetate and Sorensen's phosphate buffer mixtures, respectively, of the indicated pH values. (See Clark, W. M., "The Determination of Hydrogen Ions," 1922, 2nd edit., Baltimore, ch. vi.)

mixed with the sera of rabbits recently recovered from vaccinia the granules were rendered non-infectious; the infectivity of the granules was not neutralized by normal rabbit sera. Consideration of the results of previous work[1, 2, 3] and of this work leaves no doubt in our minds that the property of infectivity is borne by some of the particles in the washed granular material.

The washed granules have been found to become non-infectious at hydrogen ion concentrations more acid than pH = 5.2 or 5.3 (See Table I). This is in sufficient agreement with the results of Douglas and Smith,[4] who found that "diffusates" of vaccine virus from infected rabbits' testes were rapidly inactivated at reactions more acid than pH = 5.5.

We have found that infectivity could be restored to non-infectious mixtures of granules and immune serum by washing the serum-treated granules in saline or Ringer's solution.[5, 6, 7] Yet recovery of infectivity on washing was not complete; the infecting power of the rewashed granules treated with immune sera was considerably inferior to that of granules similarly treated with normal sera. Two of our experiments which show these effects most clearly are given in Tables II and III. It would seem that immune sera do produce some specific effect on the virus *in vitro,* even though it has proven difficult to demonstrate this without the aid of animal inoculation.[6, 8]

We have attempted to produce agglutination and reduction of cataphoretic velocity[9] of the four-times "washed granules" by several types of sera, as follows (a) The sera of rabbits vaccinated and revaccinated with crude commercial calf vaccine. Such sera gave pronounced precipitin reactions against bovine serum, and protected against vaccinia. These sera caused pronounced although incomplete agglutination of the washed granular material and markedly reduced cataphoretic velocity.

(b) The sera of rabbits inoculated with infectious products of

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[3] Ward, H. K., *J. Exp. Med.,* 1929, l, 31.

[4] Douglas, S. R., and Smith, W., *Brit. J. Exp. Path.,* 1928, ix, 213.

[5] Todd, C., *Brit. J. Path.,* 1928, ix, 244.

[6] Andrewes, C. H., *J. Path. and Bact.,* 1928, xxxi, 671.

[7] Long, P. H., and Olitsky, P. K., *Science,* 1929, lxix, 72.

[8] Schultz, E. W., Bullock, L. T., and Lawrence, F., *J. Immunol.,* 1928, xv, 243. See, however, Bedson, S. P., *Brit. J. Exp. Path.,* 1929, x, 364; Bedson, S. P., and Bland, J. O. W., *ibid.,* 393; Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.,* 1929, l, 673.

[9] For technic, see Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.,* 1929, xlix, 779.

commercial calf vaccine; these sera gave negative precipitin tests with bovine serum; also the sera of rabbits vaccinated with rabbit testicular or neurovirus. These sera protected against vaccinia. With the "washed granules" they gave only very weak agglutination and reduction of cataphoretic velocity. We could not be sure whether or not the agglutination and cataphoretic results obtained with these

TABLE II.
Protection from Infective Granules by Immune Sera, with Partial Recovery of Infectivity of the Same Granules After Washing.

Granules in presence of serum from:	Dilutions of Serum				
	1:4	1:16	1:64	1:256	1:1024
Vaccinated rabbit No. 1890	0	0	+	+	++++
Vaccinated rabbit No. 1892	0(?)	++ to +	++++ to +++	++++ to +++	
Revaccinated rabbit No. 2883	0	+	+++	++++ to +++	
Revaccinated rabbit No. 3024	0	trace	++	+++	
Normal rabbit No. 3179	++++ to +++	++++	++++		
Normal rabbit No. 3187	+++	++++	++++		
The above granules washed after treatment with serum from:					
Vaccinated rabbit No. 1890	+ to tr	++ to +	++ to +		++++
Vaccinated rabbit No. 1892	++	+++ to ++			
Revaccinated rabbit No. 2883	++	+++ to ++	+++		
Revaccinated rabbit No. 3024	tr	+	++ to +		
Normal rabbit No. 3179	++++ to +++	+++	+++		
Normal rabbit No. 3187	+++ to ++	+++ to ++	++ to +		

Plus marks indicate intensities of lesions resulting from intradermal injections of 0.1 cc. each. Lesions in rabbit inoculated with mixtures of granules and sera read on fifth day; lesions in rabbit inoculated with serum-treated granules after washing read on fourth day.

sera were to be regarded as very weak positive specific antivirus reactions or as negative reactions.

(c) The sera of rabbits injected with the skin scrapings of a normal calf. These sera gave positive precipitin reactions with bovine serum, but did not protect against vaccinia (See Table III). They caused partial agglutination of "washed granules" to a slightly greater degree than the sera of group b.

TABLE III.

Protection from Infective Granules by Immune Sera, with Partial Recovery of Infectivity of the Same Granules After Washing.

Granules in presence of serum from:	Dilution of Serum			
	1:4	1:16	1:64	1:256
Revaccinated rabbit No. 1890	0	0	0	tr
Revaccinated rabbit No. 1892	0	0	0	0
Normal rabbit No. 3151	++	++	++	
Normal rabbit No. 3152	++	++	++	
Anticalf rabbit No. 3132	+++	+++ to ++	+++ to ++	
Anticalf rabbit No. 3134	+	++ to +	+++ to ++	
The above granules washed after treatment with serum from:				
Revaccinated rabbit No. 1890	+ to tr	++	+++	+++
Revaccinated rabbit No. 1892	++ to +	+	+++	+++
Normal rabbit No. 3151	++++ to +++	++++ to +++		
Normal rabbit No. 3152	+++	++++ to +++	++++ to +++	
Anticalf rabbit No. 3132	+++	++++ to +++	++++	
Anticalf rabbit No. 3134	++++	++++	++++ to +++	

Lesions in rabbit inoculated with mixed granules and serum read on third day; lesions in rabbit inoculated with treated washed granules read on sixth day. "Anticalf" rabbits immunized with skin scrapings from a normal unvaccinated calf.

(d) Normal rabbit sera. These caused a trace of agglutination and reduction of velocity of "washed granules," in these reactions being slightly less positive than the sera of groups b and c but much less positive than those of group a.

The only conclusions we feel justified drawing from these *in vitro* serological tests is that the washed granular material contains calf protein in addition to the virus. This conclusion seems the more probable when one remembers that the "washed granules" are obtained by centrifugation of ground-up material which includes calf epidermis and lymph as well as vaccinia virus.

Therefore we regard the "top layer" or "washed granules" obtained from commercial calf vaccine by the method of Craciun and Oppenheimer[2] as advantageous material with which to start, but by no means as the final product, in the identification and purification of the virus.

The vaccines used were furnished us through the kindness of Dr. W. F. Elgin, of the H. K. Mulford Laboratories, and of Dr. T. M. Rivers.

4744

B. Pyocyaneus as a "Carrier" of B. Coli Bacteriophage.

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The phenomenon here reported was first observed in the course of an unsuccessful attempt to discover among a number of specimens of *B. coli* bacteriophage one that would be active upon *B. pyocyaneus*. Of 25 filtrates showing marked lysis of one or more strains of *B. coli* none could be demonstrated to have any lytic power for any of the 7 strains of *B. pyocyaneus* tested. The bacteriophage specimens were obtained from the following sources: sewage 4; urine 7; feces 15; pus 2; unknown source 1. The phages were tested on the pyocyaneus cultures both as fresh filtrates and after their lytic power for *B. coli* had been materially enhanced by repeated passage. Neither clearing of young broth cultures nor plaque formation could be demonstrated with any of the strains of *B. pyocyaneus*.

An attempt was then made with one bacteriophage specimen to bring about pyocyaneus lysis by serial passages upon cultures of the latter organism. A filtrate having marked lytic activity on several strains of *B. coli* was chosen for this test and its activity was brought to a maximum by a number of passages upon a strain of *B. coli,* S 1. This coli strain was isolated from a case of pyelitis, belonged to the slow lactose-fermenting group, gave gas on saccharose and marked hemolysis on blood agar plates. One one-hundred-millionth (10^{-8}) cc. of the lytic filtrate produced complete lysis of a young broth culture in less than 18 hours and, spread upon agar, gave numerous plaques.

Three pyocyaneus strains were used in the test. These all gave the typical abundant growth on agar with the characteristic blue-green pigments and funnel shaped gelatin liquefaction. There was a striking difference in the fermentation reactions of the 3 strains. These are given in Table I.

TABLE I.

B. pyocaneus Strain	Source	Glucose	Mannite	Lactose	Saccharose	Maltose	Milk
193	Urine	——	——	——	——	——	Coag. without acid
194	Antrum	acid	——	acid	——	acid	Coag. acid
195	Blood Culture	——	——	——	——	——	Coag. without acid

The procedure in the tests was as follows: —0.5 cc. of the lytic filtrate was inoculated into a 4-6 hour culture of the pyocyaneus strain being tested and the latter placed in the incubator over night. The next day this culture was observed closely for lysis, comparison being made with a bacteriophage-free culture of the same age. It was then filtered through a sterile Chamberland candle and 0.5 cc. of the filtrate added to a fresh 4-6 hour culture of the same strain. This was repeated daily for periods of time varying for the different strains. At no time did any evidence of lysis appear in any of the broth cultures and repeated attempts to produce plaques with the filtrates uniformly failed.

At various stages in the experiments 0.5 cc. portions of filtrate were tested upon the *B. coli* strain S 1. In these tests the most striking results were shown by the filtrates of the strongly fermenting strain 194. Filtrates up to the fortieth serial passage gave complete lysis of *B. coli* S 1, and produced abundant plaques on agar plates. The amount of filtrate necessary to bring about these results, however, gradually increased. Filtrates beyond the fortieth failed to produce complete clearing of a broth culture of S 1 though plaques were readily demonstrated with filtrates up to and including the forty-ninth. Neither lysis of broth cultures nor plaques were demonstrable with the fiftieth filtrate. However, when a broth culture of S 1 was incubated with 0.5 cc. of the fiftieth pyocyaneus filtrate, the filtrate from this coli culture could be demonstrated to contain the phage in practically its original activity.

Strains 193 and 195 of *B. pyocyaneus* showed decidedly less efficiency in maintaining the original activity of the coli bacteriophage. With one of these strains (195) 12 serial passages brought about a total loss of the phage, though some activity could be demonstrated with the 11th filtrate. With strain 193 lytic activity for *B. coli* S 1 was demonstrated up to but not beyond the 20th passage.

A comparison was made of the 24th filtrate from *B. pyocyaneus* strain 194 and the original *B. coli* phage with respect to this activity toward strains of *B. coli* other than S 1. Of the 24 strains thus tested 20 gave identical results with the two filtrates. Of the other four 2 were definitely positive with the original phage and negative with the pyocyaneus filtrate, one was completely lysed by the original and only slightly by the pyocyaneus filtrate while one was definitely positive with the pyocyaneus filtrate and negative with the original phage.

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Repair in the Paranasal Sinuses of Man Following Removal of the Mucous Membrane Lining.

HERMAN SEMENOV AND FRANK B. KISTNER. (Introduced by F. R. Menne.)

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Sufficient clinical evidence has accumulated in recent years to show that certain persistent infections in degenerated mucous membranes lining the paranasal sinuses require surgical removal. In the usual operative procedure in the antrum the diseased tissue is scraped out with a curette; or the entire membrane is removed in one piece by sub-periosteal dissection through an opening in the canine fossa (Dr. Kistner). Clinically it is observed that repair occurs with variable results. In some cases the new lining is thick and in others thin. The exact nature of the new tissue has never been determined.

In our investigations human material is employed, inasmuch as there is no available experimental animal with the surgical and pathological characteristics of sinusitis in man. At the reoperation the specimens are immediately mounted on thick paper supports and immersed in Zenker's fluid. The tissues are dehydrated in alcohol, cleared in cedar oil, imbedded in paraffin and stained with hematoxylin-eosin.

Repair After Part of the Mucous Membrane is Removed:

1. Repair in a Small Area—Approximately one square centimeter was cut from the nasal wall of the antrum in the usual gland-bearing portion of the mucous membrane. Four months later the healed area with some adjacent membrane was removed. Microscopic sections show a layer of ciliated columnar pseudo-stratified epithelium resting on white fibrous scar tissue. No submucous glands are found.

2. Repair of a Large Area—About one-third of the mucous membrane was removed from 2 cases. Two years later the sinuses were reoperated and the entire antrum lining removed. Microscopic sections show the *original portion* of the membrane containing the normal histologic structures as follows: (1) A layer of pseudo-stratified ciliated columnar epithelium; (2) a superficial spongy layer of areolar connective tissue with large tissue spaces; (3) tubo-alveolar mucous and serous glands in the layer of loose connective tissue; (4) an orderly arrangement of branching arteries and arterioles in definite layers; (5) a definite periosteal layer of compact connective tissue. While the *repaired portion* of the lining consists of a thick mass of fibrous scar tissue covered for the most part by an indifferent layer of cubical epithelium which is stratified in some areas and entirely absent in others, an irregular vascularization of scar tissue is present. The usual histological structure of antrum mucous membrane is entirely absent. No glands are seen. No loose areolar tissue with large tissue spaces is seen. (See photomicrograph.)

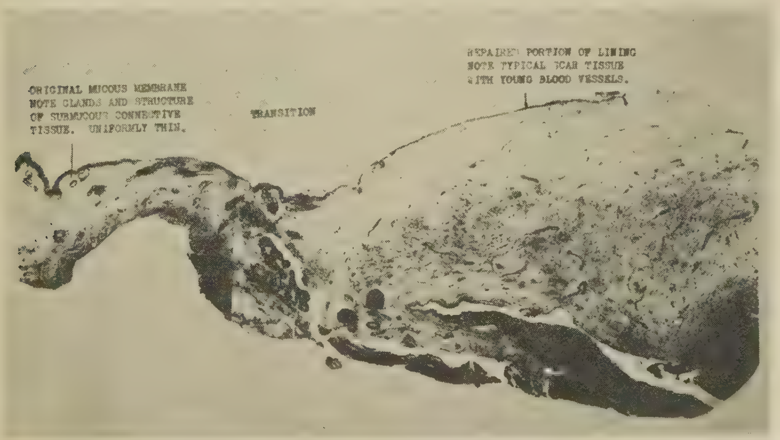

Fig. 1.

-Photomicrograph of a healed portion of sinus lining from the antrum of a human being, showing the original lining on the left, the transition point at which repair started in the center and the thick new scar tissue lining on the right.

Repair After the Entire Mucous Membrane is Removed:

1. Epithelization from the Nasal Mucous Membrane—After complete subperiosteal exenteration of the lining through the canine fossa a counter-opening is made in the nasal wall of the antrum to permit drainage. A flap of nasal mucous membrane several millimeters in width is usually brought into the antrum to cover the floor of the counter-opening. Two cases were examined, one 8 months and the second 18 months after the primary operation. Repair produced a great deal of bony thickening in the walls of the sinuses. Whereas the original antrum lining is easily peeled from the bone, the new lining is only torn away with difficulty. Spicules of bone are found imbedded in the scar tissue of the new lining. The healed antrum is lined by a thick, dense, firm layer of scar tissue covered by stratified columnar epithelium. The normal antral membrane contains only a few glands on the nasal wall, but the membranes obtained after repair with the nasal flap contain many more mucous and serous glands than the original lining. The glands are derived from the nasal flap and are buried in the firm scar tissue. The white fibrous scar tissue contains fibroblasts in the superficial portion, and dense wavy strands deeper in the lining. There is no loose areolar connective tissue.

Recently, Knowlton and McGregor,[1] working on 3 dogs curetted portions of the mucous membrane from the recess in the nasal cavity and observed complete epithelial regeneration in one dog at 3 months and gland regeneration in another dog at 5 months.

2. Epithelization from the Oral Mucous Membrane—In one case, after sub-periosteal exenteration, the opening through the canine fossa was kept patent. After 6 months the antrum was lined with a firm membrane composed of stratified squamous epithelium as recently recorded by one of us (Kistner[2]). This lining is similar to that of the oral cavity from which it was derived.

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[1] Knowlton, Charles D., and McGregor, G. W., *Arch. Otolar.*, 1928, viii, 647.

[2] Kistner, F. B., *Ann. Otol. Rhin. and Laryn.*, 1929, xxxviii, 977.

4746

Hemodynamics of Arteriosclerosis Influence of Elastic Factor on Circulation.

GEORGE FAHR, JAY C. DAVIS AND RUSSEL SPITTLER.

From the University of Minnesota Medical School.

Using a modified heart-lung preparation of Starling, with the arterial cannula inserted in the brachial cephalic artery in the first experiments, and in the later experiments in the arch of the aorta, the following experiments were done.

In drawing conclusions as to the external work of the heart, we consider that our experiments establish the diastolic volume of the heart as an index of the energy consumption of the heart and therefore a measure of the work of the heart, provided the heart muscle function remains as constant throughout the period under consideration. Only one experiment is given here.

Calibration of elastic coefficient in terms of percentage volume increase of arterial system to mm. Hg. increase pressure or mm. Hg. increase in pressure/% increase in volume. This is the "effective" elastic coefficient or rigidity of arterial system.

Without Air Chamber or Arteriosclerosis. Content of "effective" arterial system = 210 cc. 2 cc. blood injected into system gives a rise of 56 mm. Hg. in pressure.

2/210 = 0.95% increase in volume for 56 mm. Hg., or 1% increase in volume for 59 *mm. Hg.* A. V. Hills normal artery between 80-120 mm. Hg. showed 6.6% increase in volume for 40 mm. Hg., or 1% increase in volume for 6 *mm. Hg.* Ergo in this system without air chambers and representing extremely severe arteriosclerosis, there is a rigidity 9.8 x normal "mean" artery of A. V. Hill at normal pressure.

With Air Chamber or Normal. Content of arterial system = 240 cc. 20 cc. blood injected into system gives a rise of 27 mm. Hg. 20/240 = 8.3% for 27 mm. Hg., or 1% increase for 3.3 mm. Hg. Ergo rigidity is ½ that of A. V. Hill "mean" normal artery. Rigidity without air chamber or in arteriosclerosis = 18 x that with air chambers or in normal.

Calibration of Cardiometer.

$$20\text{ cc.} = \left.\begin{array}{r}13\text{ mm.}\\13\text{ mm.}\\12\text{ mm.}\end{array}\right\}12.7\text{ mm.}$$

Aortic cannula = 9 mm.
= 8 mm. at narrow point

	Without Air Chamber (Arteriosclerosis)		With Air Chamber (Normal)	
Sys. B.P.	= 140	2567 mm. Hg.	= 80	38 mm. Hg.
Dias. B.P.	= −116		= 42	
Stroke Vol.	= 13.0 cc.		= 13.0 cc.	
Minute Vol.	= 1014 cc.	Heart rate 78.	= 1014 cc.	
Circul. Vol.	= 686 cc.		= 692 cc.	
Coronary flow	328 cc.		= 322 cc.	

Diastolic volume is 1 cc. less without air chambers than with air chambers.

Sys. B.P.	= 134	250? mm. Hg.	= 80	38 mm. Hg.
Dias. B.P.	= −116		= 42	
Stroke Vol.	= 14 cc.		= 13.4 cc.	
Minute Vol.	= 1064 cc.	Heart rate 76.	= 1018 cc.	
Circul. Vol.	= 693 cc.		= 706 cc.	
Coronary flow	371 cc.		= 312 cc.	

Diastolic volume is 0.5-1.0 cc. less without air chambers than with air chambers.

Sys. B.P.	= 136	238? mm. Hg.	= 90	52 mm. Hg.
Dias. B. P.	= −102		= 38	
Stroke Vol.	= 14.3 cc.	Rate 74.	= 13.8 cc.	
Minute Vol.	= 1058 cc.		= 1021 cc.	
Circul. Vol.	= 680 cc.		= 686 cc.	
Coronary flow	378 cc.		= 335 cc.	

Diastolic volume in arteriosclerosis is 0.75 cc. less than in normal.

Sys. B.P.	= 128	238? mm. Hg.	= 78	34 mm. Hg.
Dias. B.P	= −110		= 44	
Stroke Vol.	= 14.5 cc.	Heart rate 72.	= 14.5 cc.	
Minute Vol.	= 1015 cc.		= 1015 cc.	
Circul. Vol.	= 680 cc.		= 706 cc.	
Coronary flow	335 cc.		= 309 cc.	

Diastolic heart volume 0.75 cc. less in arteriosclerosis.

In all other experiments, the diastolic pressure remained above the base line.

Throughout the course of the experiment, lasting 2 hours, heart volume in diastole is approximately 3 cc. less at end than at beginning of experiment:

Minute Vol. = Total output of left ventricle calculated from ½ cardiometer change in systole multiplied by heart rate.

Circul. Vol. = Volume of flow as measured with stop watch and calibrated measuring cylinder on blood flowing out of venous system into venous reservoir.

Stroke Vol. = Output of one ventricle. This value is obtained by multiplying the height of the cardiometer curve in millimeters by 20 and dividing by 2 times the increase in height of the cardiometer tracing when 20 cc. are forced into the cardiometer system from a record syringe connected to the system.

Conclusions: 1. The external work of the heart is not increased when the "effective" rigidity of the arterial system is increased 10 times the normal, provided the lumen of the arterial system remains the same and the rigidity of the venous system remains normal and, therefore, much lower than the rigidity of the arterial. 2. The minute volume of blood flow in the systemic arterial system is not decreased more than 5% when the "effective" coefficient of volume elasticity of the arterial system is increased 10 times the normal, provided the lumen of the arterial system remains the same and the rigidity of the venous system remains normal and, therefore, much lower than the rigidity of the normal arterial system. 3. Apparently at times the systolic ejection time is lengthened and the diastolic filling time of the left ventricle decreased when the rigidity of the arterial system is increased greatly, thus causing slightly decreased diastolic volume of the ventricles and decreased stroke volume. The coronary flow is slightly decreased by the increased length of systole. The external work of the heart is slightly decreased also. These conclusions must be confirmed by optical recording and more accurate stroke volume measurements.

4747

Presence of the Morning Alkaline Tide in a Case of Achlorhydria.

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In previous communications from this clinic it has been stated that the urinary alkaline tide is primarily due to the secretion of hydrochloric acid by the stomach, for the tide was absent in most cases of achlorhydria.[1] This observation has been confirmed by Ackman[2] and Davies.[3] Further study showed that it was necessary to distinguish between alkalinity developing during the day-time and that shown immediately after awakening,[4] for this latter change is frequently seen in patients where hydrochloric acid is not found in the stomach, and is probably due to the respiratory adjustment of the subject to waking conditions.[5] Recently we have studied a patient with achlorhydria who showed a tide exactly resembling those found in normal subjects. It seems desirable for us to report this case because the absence of hydrochloric acid was found after histamine had been injected—a method giving as complete proof of functional disability of the stomach as can be obtained. In many cases where a urinary tide has been present, but hydrochloric acid apparently was absent from the gastric juice, further gastric studies have failed to confirm the abnormal finding of the first one,[6] and it has been claimed by some that the presence of a satisfactory alkaline tide furnishes definite proof that the stomach secretes hydrochloric acid.

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[1] Munford, S. A., and Hubbard, R. S., *J. Am. Med. Assn.*, 1926, lxxxvii, 922.

[2] Ackman, F. D., *Canadian Med. Assn. J.*, 1925, xv, 1099.

[3] Davies, D. T., *Brit. J. Exp. Pathol.*, 1929, x, 1.

[4] Hubbard, R. S., *J. Biol. Chem.*, 1929, lxxxiv, 191.

[5] Leathes, J. B., *Brit. Med. J.*, 1919, ii, 165.

The patient was a man 46 years old who was a mechanical engineer by profession. He had occasional periods of mild diarrhoea, and symptoms which were somewhat suggestive of the presence of a peptic ulcer. The stomach emptied rapidly, as frequently is the condition in cases of achlorhydria. Gastric analyses were carried out, and the reaction of successive samples of morning urine determined. A discrepancy between the results of the 2 tests was noted, and further studies were accordingly made.

Free hydrochloric acid was not found in the gastric contents, even when histamine in the maximal amounts used in such tests was given. The case therefore should be considered as one of achlorhydria.

TABLE I.

Studies of the alkalinity of the urine.

Period spent	Morning meal	Reaction of urine specimens.						
		Time.						
		to 7	7-8	8-9	9-10	10-11	11-12	12-1
		pH	pH	pH	pH	pH	pH	pH
up	taken	5.1	5.7	6.6	7.3	8.0	7.5	6.8
abed	taken	5.1	5.5	5.9	6.5	7.5	7.3	6.7
abed	omitted	4.9	5.1	5.3	6.6	6.9	7.6	6.8
			Time.					
			11-1	1-3	3-5	5-7	7-9	
			pH	pH	pH	pH	pH	
Afternoon tide			6.5	5.3	5.2	5.2	5.2	

The table shows the urinary reaction determinations made upon this patient. Atypical morning alkaline tide was present whether a meal was fed or omitted. Exactly similar results have been obtained on various normal subjects.[7] The tide was also demonstrated when the patient was in bed, thus probably reducing respiratory changes to a minimum, as well as when he was up and moderately active. The afternoon tide, which seems to be present in most normal subjects,[8] was lacking.

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[6] Hubbard, R. S., *Clifton Med. Bul.*, 1928, xiv, 7.

[7] Hubbard, R. S., and Steele, T. M., *J. Biol. Chem.*, 1929, lxxxiv, 199.

The morning tide observed was almost certainly not produced by the secretion of hydrochloric acid by the stomach, nor was it connected with the ingestion of a meal. The experiments also seem to show that it was not caused by respiratory adjustment to waking conditions. It is possible that in this subject there is an unusual persistence of the "habit" alkali-acid rhythm implied by the work of Muschat[9] and others. It is known that the rhythm sometimes persists when the stimulus to gastric secretion is absent, and here it may have persisted when acid secretion by the stomach had wholly ceased. Whether this explanation is or is not adequate, such a case as this, where an absence of hydrochloric acid is clearly demonstrated in a patient who shows a typical alkaline tide, makes necessary a somewhat conservative attitude towards the clinical interpretation of alkaline tide studies.

4748

Effect of Metals Purified by Electrolytic Deposition on Hemoglobin Regeneration in Anaemic White Rat.

GEORGE T. LEWIS, THEODORE E. WEICHSELBAUM AND JOSEPH L. MCGHEE. (Introduced by D. A. McGinty.)

From the Laboratory of Biochemistry, Emory University, Ga.

Recent work by Hart, Steenbock and others[1] and Myers and Beard[2] has given rise to opposing results as to the value of metals other than copper in the regeneration of hemoglobin in the anaemic white rat. The suggestion has been made[3] that these differing results were due to the fact that the work was carried out in 2 rather widely separated localities. It has seemed to us that the publication of our results obtained in a third and entirely different section of the country might be of value as bearing on this point.

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[8] Hubbard, R. S., Munford, S. A., and Allen, E. G., *Am. J. Physiol.*, 1924, lxviii, 149.

[9] Muschat, M., *J. Clin. Invest.*, 1926, ii, 245.

[1] Waddell, J., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1929, lxxxiv, 115.

[2] Myers, V. C., and Beard, H. H., *J. Am. Med. Assn.*, 1929, xciii, 1210.

[3] Discussion by Drs. Hubbard, Steenbock and Myers, *J. Am. Med. Assn.*, 1929, xciii, 1212.

The rats used were from a local laboratory strain, originally a Wistar Institute stock. The milk used came entirely from one cow, was milked in an enamelled vessel and brought to the laboratory in glass bottles, with the result that there was no contact with metal previous to feeding. This precaution was found to be necessary since neither raw nor pasteurized milk bought on the open market sufficed to produce an anaemia in the white rat, although it comprised the sole diet of the animals for a period of 6 months.

Several attempts were made to obtain iron, free from traces of other metals, but electrolytic deposition was the only method producing a sample of iron which does not allow a regeneration of hemoglobin in anaemic white rats. Whether or not it may prolong the life of the animal,[4] we are not at this time prepared to state. The method used for the electrolytic deposition of iron was that of Classen and v. Reis.[5] Copper was prepared electrolytically by the method of H. Sand,[6, 7] and cobalt by that of Denso.[8] The preparation of manganese electrolytically in the form of the oxide, free from traces of copper, by the method of Engel,[9] was unsuccessful and, therefore, the manganese solution used in our experiments was prepared from manganese sulfate (C. P. Baker's) by subjecting it to the usual scheme of qualitative analysis, and isolating the manganese as the chloride. The acid used to remove the metals from the platinum electrodes was Mallinckrodt Reagent Quality. Iron

TABLE I.

No. of rats	Metal fed	Hemoglobin (gm. per 100 cc.)			
		6/5	6/13	6/27	7/16
9	Fe.	5.5-8.5 Av. 6.6	5.0-7.0 Av. 6.4	5.0-7.5 Av. 6.2	
8	Fe.+Mn.	4.0-7.0 Av. 5.0	5.0-5.5 Av. 5.2	4.25-6.0* Av. 5.2	7.0-12.0 Av. 9.7
8	Fe.+Co.	3.0-4.5 Av. 3.6		All readings = 3.5*	6.0-11.5 Av. 7.6
7	Fe.+Cu.	4.25-6.75 Av. 5.2	8.0-11.5 Av. 9.7	8.0-15.5 Av. 12.0	

* Changed to Fe.+Cu. on the day following this reading.

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[4] Goerner, A., *J. Lab. Clin. Med.*, 1929, xv, 119.

[5] Classen, A., and v. Reis., Ber., 1881, xiv, 1622.

[6] Sand, H. J., *J. Chem. Soc.*, 1907, xci, 373. (Quantitative analysis by Electrolysis, A. Classen-Hall. New York, 1913.)

[7] Sand, H. J. S., *Z. Elektrochem.*, 1907, xiii, 326. (Quoted by A. Classen. See 6.)

[8] Denso, P., *Z. Electrochem.*, 1903, ix, 469. (Quoted by A. Classen. See 6.)

[9] Engel, H., *Z. Elektrochem.*, 1895, ii, 413, 1896, iii, 286, 305. (Quoted by A. Classen. See 6.)

was fed as the nitrate in amounts of 0.5 mg. of metal per rat per day; copper, cobalt and manganese as the chlorides in amounts of 0.1 mg. of metal per rat per day.

Hemoglobin determinations were made by the method of Newcomer.

The results of the experiments are briefly summarized in Table I.

It is evident, therefore, that under the conditions of our experiments *pure* iron, fed alone, does not cause a regeneration of hemoglobin in anaemic white rats. Copper is effective in the regeneration when fed together with iron, while manganese and cobalt fed under the same conditions are without effect.

4749

Effect of Placental Extracts on Ovarian Stimulating Properties of Anterior Hypophysis.*

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From the Department of Gynecology and Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee.

The interrelationships between the hypophysis and the ovary have been studied extensively. Changes in the ovary following the administration of anterior lobe substance and extracts have been described by Goetsch,[1] Evans,[2] Zondex and Ascheim,[3] and others. Erdheim and Stumme[4] called attention to changes in the hypophysis during pregnancy. Zondex and Ascheim have demonstrated, in the urine of pregnant women, the presence of a hormone which has all the ovarian growth properties of the anterior hypophysis, and have utilized this in their test for pregnancy. Fels[5] found the same hormone in the blood of pregnant women. Recently Engle and Smith[6] have demonstrated that the amount of ovarian stimulating substance in the hypophysis of the guinea pig varied during the sexual cycle, and was much diminished during the time the vagina was open. Evans and Simpson[7] further showed that this substance was increased by castration, and was present in larger amounts in males than in females.

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* Part of the animals used in these experiments were drawn from the colony supported by the Commonwealth Fund.

[1] Goetsch, Emil, *Johns Hopkins Hosp. Bull.*, 1916, xxvii, 29.

[2] Evans, Herbert M., *Harvey Lectures*, 1924, xix, 212.

[3] Zondex, B., and Ascheim, S., *Arch. f. Gyn.*, 1927, cxxx, 1.

[4] Erdheim, J., and Stumme, E., *Beitg. z. Path. Anat. u. z. Allg. Path.*, 1909, xlvi, 1.

[5] Fels, Erich, *Wien. Klin. Woch.*, 1928, xli, 1225.

[6] Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1929, xliii, 239.

From the foregoing work the following facts are known: (1) the hypophysis has a powerful action on the ovary; (2) the ovary affects the hypophysis; (3) there is a change in the hypophysis during pregnancy. With these conclusions in view, it seemed reasonable to believe that the ovarian growth stimulating properties of the anterior hypophysis could be increased by the injections of placental extracts which contain a high concentration of oestrin.

Our experiments tend to support this belief. Adult female rats, of approximately the same weight, were castrated, in order to exclude any cyclical variation, allowed to recover for 2 weeks, and then given a series of injections of a commercial alcoholic extract of human placenta which had been carefully standardized. The injections were given for 6 days and varied from 5 to 25 rat units. The animals were sacrificed and the anterior lobe of the hypophysis implanted into infantile mice of 8-12 gm. weight. (In none of these was the vaginal orifice open at the beginning of the experiment.) Control mice were implanted with hypophyses from castrated rats which had not been injected with the extract. Each mouse received 2 hypophyses which were implanted on successive days, and the mice were sacrificed on the fourth day of the experiment.

Our series of experimental mice included 32 animals, and there were 16 controls (*i. e.,* mice receiving hypophyses from rats which had been castrated but had not received placental extract.) The most noteworthy gross difference was the uniformly larger size of the ovaries in the experimental animals, the average difference being approximately in the ratio of 5 to 2. In addition, in the experimental animals, the bursae were enormously distended with fluid which often contained blood. In both series there were many ripe follicles and maturing corpora lutea; these were more numerous in the experimental animals and often contained blood. From 6 to 8 hemorrhagic follicles were found in the ovaries of the experimental animals, whereas only 2 to 3 at most were seen in the controls. Thus, while the changes in the experimental series were in general similar to those in the controls, they were markedly exaggerated.

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[7] Evans, Herbert M., and Simpson, Miriam E., *Anat. Rec.*, 1927, xxxv, 36.

4750

Effect of a Diet Low in Salts on Oestrous Cycle of Albino Mouse.*

J. M. WOLFE. (Introduced by R. S. Cunningham.)

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee.

In 1922 Evans and Bishop[1] found that a diet poor in salts had a definite effect on the oestrous cycle of the white rat. They found that on such a diet the experimental animals reached maturity later than their litter-mate controls and that only 15% of the oestrous cycles of these animals were of the 4 or 5 day length as opposed to 48% of those of the control animals.

We have carried out similar experiments on the white mouse. The animals used were about 60 days of age. Vaginal smears were made daily for 3 weeks, and all animals not showing cycles of 4 to 5 day length were discarded. They were then divided into experimental and control groups.

The following experimental diet was used: Casein 31%, dextrin 41%, Crisco 20%, cod liver oil 3%, yeast 5%.

The control animals received the same diet, with the exception that salts were fed at a 7% level and the dextrin reduced to 35%.

There was a very definite change in the oestrous cycles of the experimental animals, only 25% of the cycles being of the 4 or 5 day length, while many of the long cycles were 12 to 20 days in length. In the control group, 70% of the cycles were 4 or 5 days in length and in the remaining cycles, only a few were over 7 days in length.

The experimental animals grew at a slightly lower rate than the control animals. The difference at the peak of the experiment was 13.75%. In all cases, however, the latter exhibited a rather sleek coat, as contrasted with the coarse, roughened fur of the experimental mice.

It is evident from these experiments that the oestrous cycle was affected much more than the growth ability, indicating that the oestrous cycle is a much more delicate indicator of the well-being of the animal than is the growth curve.

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* This work has been supported by a grant from the Commonwealth Fund.

[1] Evans, H. M., and Bishop, K. S., *J. Metabolistic Res.*, 1922, i, 335.

4751

The Effect of Ergotamine on Intestinal Motility.

WILLIAM SALANT. (With the technical assistance of H. Nagler and Leon Herbert Ehrlich.)

From the Biological Laboratory, Cold Spring Harbor, L. I., N. Y.

According to Mendez[1] ergotamine produced depression of motility in the isolated intestine of the rabbit. Later Issekutz and Leinzinger[2] reported that it was without effect on the intestine of these animals. More recently Rothlin[3] stated that ergotamine produced no effect in some rabbits and caused decreased motility in others. He also reported that in experiments with ergotamine on the intestine *in situ* of dogs his results were negative. Planneles[4] observed in the guinea pig that the isolated intestine of these animals was depressed by ergotamine. Thienes[5] recently carried out experiments on the isolated intestine of the guinea pig, rabbit and cat with adrenalin and low concentrations of ergotamine (1:250,000 and 1:500,000). He made no statement, however, of the action of ergotamine but a tracing of the intestine of a rabbit shows that no change in motility occurred as a result of the treatment with ergotamine. In the course of studies on the effect of intestinal motility[6] in which ergotamine was occasionally employed, it was noticed that the intravenous injection of the substance stimulated peristalsis in cats under urethane anesthesia. More extensive observations carried out since fully corroborated our earlier findings, and we were able also to show that the increase in the motor functions was in some cases very striking. These studies, which were carried out by the Trendelenburg method, on the intestine *in situ* were extended to experiments on the isolated intestine of the cat, suspended in oxygenated Locke's solution and kept at body temperature. Ergotamine in concentration of about 1:200,000 to 1:500,000 produced depression, the tonus being slightly decreased and the rhythmic movements were abolished or greatly diminished in force and frequency. As the results might have been due to a substance liberated when ergotamine was injected into the circulation, blood was obtained from treated animals, defibrinated, added to an equal volume of Locke's solution and the action of ergotamine was then tested. The results after adding ergotamine were not constant, showing in some cases stimulation, in some depression. But in experiments in which defibrinated blood from untreated cats was mixed with Locke's solution ergotamine produced the same effects. Indeed, our tracings in one series of experiments showed, on the contrary, that moderate but distinct stimulation was produced by ergotamine

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[1] Mendez, *J. Pharmacol. and Exp. Therap.*, 1927, xxxii, 451.

[2] Issekutz and Lenzinger, *Arch. Exp. Path. u. Pharmakol.*, 1928, cxxviii, 165.

[3] Rothlin, *J. Pharmacol. and Exp. Therap.*, 1929, xxxvi, 657.

[4] Planneles, *Arch. Exp. Path. u. Pharmakol.*, 1929, cv, 38.

[5] Thienes, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 501.

[6] Salant and Brodman, *J. Pharmacol. and Exp. Therap.*, 1929, xxxvii, 55.

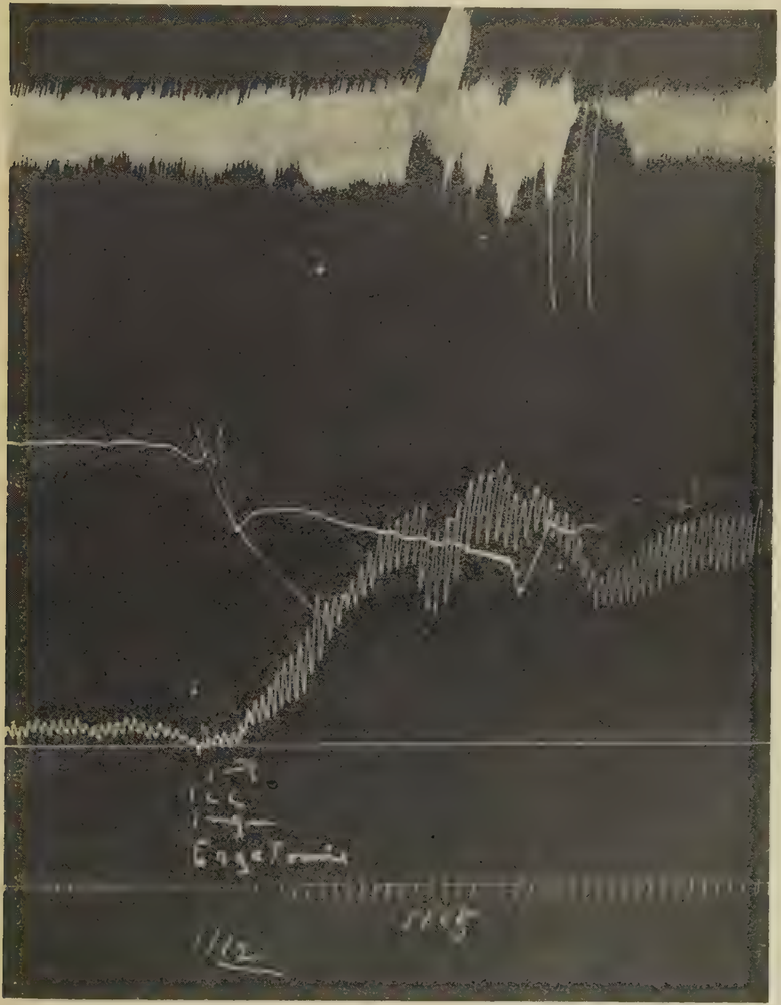

FIG. 1.

Experiment 773, female cat, wt. 2.8 K., urethane anesthesia. One mg. ergotamine injected intravenously stimulated the intestine. Tracings from above downward show respiration, blood pressure, intestinal movement. Time in tenth seconds and minutes.

in concentration of 1:250,000 to 1:50,000 or even 1:25,000. The blood probably contained, therefore, a substance (or substances) that modified the action of ergotamine.

We are very much indebted to Mr. Eugene Marti of the Sandoz Company for a generous supply of ergotamine, gratis, used in this and other experiments by the writer.

4752

The Reversal of Action of Ergotamine by Calcium and Changes in C_H.

WILLIAM SALANT AND HAROLD NAGLER.

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As stated in the preceding communication,[1] the response of the intestine to ergotamine has been studied in different animals. No observations have been made, however, to determine whether changes in ionic content of the surrounding medium modified the action of ergotamine. We, therefore, undertook the investigation of this problem and selected the intestine of the rat for the test object. As far as we know, no studies with ergotamine on the intestine of this animal have been reported. The experiments of Rosenmann,[2] however, are of interest in this connection. He observed that ergotoxin produced stimulation in the isolated intestine of the rat.

Our experiments were carried out by means of the Magnus method, segments of different parts of the intestine being suspended in oxygenated Locke solution maintained at almost uniform temperature, usually 37.5° C. The variations which sometimes occurred seldom exceeded 0.2° C. We found that concentrations of 1:100,000 and sometimes 1:1,000,000 ergotamine in Locke solution with a pH of about 7.2 or 7.3 and containing 0.014% calcium chloride, produced a considerable decrease of tonus, the relaxation being more marked in the ileum and colon than in the duodenum. The rhythmic movements, however, were usually stimulated, especially the amplitude. When the amount of calcium in the solution was increased to 0.028 or 0.056% a striking change in the action of ergotamine occurred when the H-ion concentration was decreased below neutral-ity, especially when it was around pH of 6.5. The same amounts of ergotamine now caused a very marked increase of tonus which occurred within a few seconds after it was added to the solution. The duration of the effect varied in different experiments, but it usually lasted several minutes or longer. The rhythmic contractions were also increased in some cases, but they occasionally remained unchanged. The results obtained were different when the pH was raised to 7.5 or 8.0, the calcium content being 0.028 to 0.056% or more. The same amounts of ergotamine as before caused little stimulation and often failed to produce any change or even caused moderate depression of the intestine. We also made the observation

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[1] Salant, William, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, —?

[2] Rosenmann, *Z. f. i. ges. Exp. Med.*, 1922, xxix, 334.

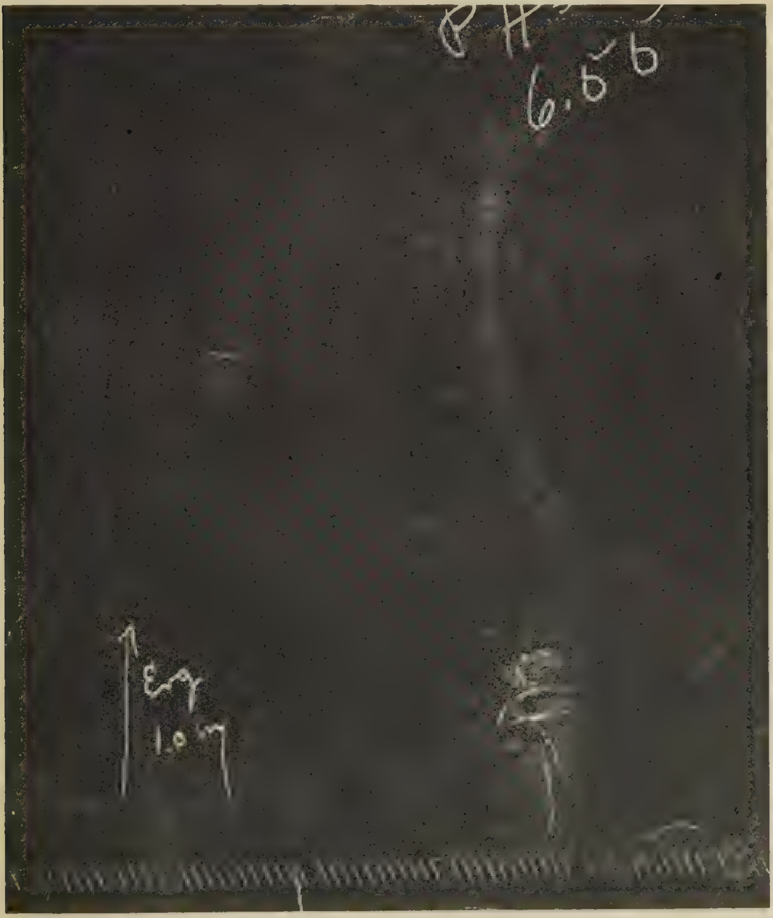

Fig. 1 A

A. Duodenum of rat in Locke's solution containing 0.028% calcium chloride, pH 6.55, Ergotamine 1:100,000 produced stimulation.

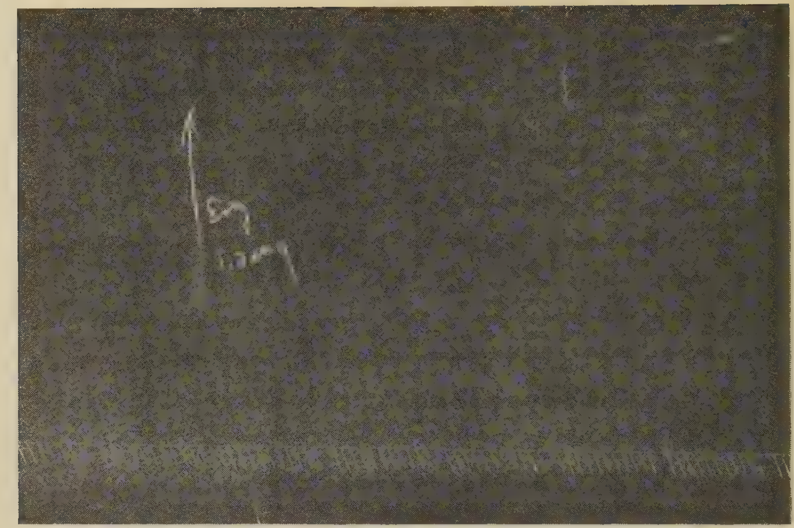

FIG. 1 B

B. Duodenum of rat in Locke's solution containing 0.014% calcium chloride, pH 6.78, Ergotamine 1:100,000 produced depression.

that the response was different in the duodenum and ileum. Higher concentration of calcium—about 0.056% or more—and smaller amounts of ergotamine were required to produce reversal by ergotamine similar to that obtained in the duodenum.

4753

Anterior Lobe of the Pig and the Monkey Ovary.

CARL G. HARTMAN. (Introduced by H. D. Senior.)

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The Smith and Engle phenomenon, first demonstrated in the rat, has been extended to other laboratory mammals. E. Allen induced sudden stimulation of the ovaries and genital tract in an immature rhesus female by means of implants of anterior lobe from 3 spayed females of the same monkey species. He also found that dog hypophysis had no effect.[1] It is, therefore, of interest to report a striking effect of pig anterior lobe upon a non-ovulating and amenorheic monkey.

A preliminary experiment was done with the glands implanted whole and intact. Female No. 22 of the Carnegie Colony of rhesus monkeys had been running irregular cycles as follows: 82, 98, 98, 25, 54, 91 days. These were her only menstrual periods; but minor cycles were reflected in the curve of vaginal desquamation. She was, moreover, blind, with complete optic atrophy, the sequel of a disease a year before the experiment. Laparotomy June 4, 1929, 8 days after the last bleeding of 5 days' duration, showed an infantile uterus and tiny ovaries absolutely devoid of visible graafian follicles, corpora lutea, or corpora albicantia. Two whole interior lobes of castrated male pigs were implanted retroperitoneally at the time of the laparotomy; 2 on June 5, intramuscularly, and again 2 on June 6. The animal was killed June 10 and embalmed with Regaud's fluid: uterus of middle finger size (15x10 mm.); ovaries 9x6.8x6 and 9x6.2x6 mm., with follicles of pin-head and mustard-seed size. The effect of the implants was noticeable but not striking.

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[1] Allen, E., *Anat. Rec.*, 1928, xxxix, 315.

An experiment with female No. 31 gave unequivocal results. This rather vigorous female of about 4000 gm. weight passed through the following menstrual cycles: 28, 28, 22, 19, 65, 31, 35, 21, 57, 45 days. She had one spell of sickness, which rendered her temporarily blind. She only partially recovered, for she continued to strike her head against the wire cage causing a scalp wound that refused to heal. May 20, 1928, a few red blood cells were seen in the vaginal lavage, her last menstruation. It was apparent, however, that the last several uterine bleedings were unaccompanied by follicular growth, a diagnosis made from outward signs, corroborated by laparotomy June 10, when the ovaries were found to be of minimal size for an adult animal (8x5x3 mm.) and the uterus the size of a little finger. At this operation the anterior lobes of 2 adult castrated pigs from the slaughter house were placed retroperitoneally and crushed with a mosquito forceps. On each of 3 successive days thereafter 2 further glands of the same kind were placed in the rectus muscles and crushed. Twice the glands were fresh and warm; twice they had been on ice 3 to 4 hours. June 17, four days after the last implant, the vaginal lavage contained red blood cells and their appearance is interpreted as the counterpart of the intermenstrual bleeding in the monkey, the proestrous bleeding of the dog. June 19 the animal was again laparotomized for inspection of the organs in the living state. The ovaries were found to be enormous, with the entire surface studded with graafian follicles 3 to 4 mm. in diameter, bluish in color from their contents of clear liquor folliculi. The uterus was much enlarged and vascular and practically round. After embalming (Regaud's fluid) it measured

16 mm. in diameter on a photograph; the ovaries after removal from the injected animal measured 13x12x10 and 14x12x10 mm., the largest ever seen in any rhesus female at any time, even when containing a corpus luteum of maximum size. The increase in size of the ovaries was due to the growth of numerous graafian follicles. The mammary glands were removed, stained, and mounted whole. They showed a considerable amount of proliferation and are comparable to the stage attained by the specimen shown by E. Allen.[2] Allen's specimen was stimulated by injection of liquor folliculi direct.

The next step will be a study of the relation of the Smith-Engle effect to the menstrual process, for it now appears certain that the follicular apparatus has nothing to do with menstruation, the cause of which is to be sought outside the ovaries.

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Effect of Anesthesia on Artificial Production of Pseudopregnancy in the Rat.*

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The condition of pseudopregnancy in the rat was first described by Long and Evans.[1] These authors demonstrated that pseudopregnancy could be produced artificially by the introduction of a small glass rod into the cervical canals at the time when the animal was in stages 1, 2 or 3 of the oestrous cycle, as specified by them. Wang[2] corroborated the findings of Long and Evans and recently Slonaker[3] has made additional contributions to the subject.

Long and Evans showed that there was no direct nervous connection between cervix and ovary. Slonaker suggests that a substance, most likely to be found in the vaginal or uterine mucosa, is responsible for the continued action of the corpora lutea in pseudopregnancy and possibly in pregnancy. He further suggests that this substance could act either directly on the corpora lutea or through some intermediary agency, possibly the anterior part of the pituitary gland. It has been shown by Smith,[4] Smith and Engle,[5] Evans and Simpson[6] and others that there are one or more substances secreted by the anterior portion of the pituitary body which influence the growth of follicles and the formation of corpora lutea of the ovary.

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[2] Allen, E., *Contrib. to Embryology,* 1927, xix, Fig. 51.

* Assisted in part by a grant from the National Research Council, Committee on Problems of Sex.

[1] Long, J. A., and Evans, H. M., *Mem. Univ. Calif.,* 1922, vi, 82.

[2] Wang, G. H., *Comp. Psychol.,* 1923, Mon. II, Serial No. 6, 1.

[3] Slonaker, J. R., *Am. J. Phys.,* 1929, lxxxix, 406.

We wish to report results obtained when rats in the first 2 stages of the oestrous cycle are anesthetized for a short time with ether, nitrous oxide, or ethylene, and the cervical canals then stimulated by a small glass rod, as compared with controls which were not anesthetized. The rats used in this study were all healthy and sexually mature. A number of the animals were selected from the stock cages at the time when they were in oestrum, without first determining the regularity of their cycles. A second group was used in which the animals had been subjected to the vaginal smear method for a period of weeks and those showing regular oestrous cycles were selected for the experiments.

In these experiments the animals were subjected to the anesthetics until there was a complete loss of tonus of the skeletal muscles, after which they were removed from the anesthetizing chamber, a nasal speculium inserted in the vagina, and the cervical canals stimulated. When ether was used the animals were almost completely out from under the anesthetic within 2 or 3 minutes while with nitrous oxide and ethylene the recovery was much more rapid. Examination of

TABLE I.

	No. of Cases	Pseudopregnancy			
		No. Cases	No. which failed	%	Combined % (A and B)
(A) No anesthesia (Normal)	12	7	5	58.3	68.8
(B) No anesthesia (Normal)	33	24	9	72.7	
(A) Ether anesthesia	14	1	13	7.1	10.4
(B) Ether anesthesia	34	4	30	11.7	
(A) Nitrous oxide anesthesia	12	2	10	16.6	21.7
(B) Nitrous oxide anesthesia	20	5	15	25.0	
(A) Ethylene anesthesia	7	3	4	42.8	33.3
(B) Ethylene anesthesia	20	6	14	30.0	

(A) Rats on which no previous vaginal smears had been taken.
(B) Rats on which vaginal smears had been taken and which had regular oestrous cycles.

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[4] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 131.

[5] Smith, P. E., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 561.

[6] Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, xci, 1337.

the table shows that the percentage of animals becoming pseudopregnant when nitrous oxide and ethylene were used is higher than those anesthetized with ether. This difference may be due to the difficulty of producing an anesthesia with nitrous oxide and ethylene which lasts long enough adequately to carry out the procedure of stimulating the cervical canals under the conditions described above. Nevertheless the important point is that the data do show that the percentage of pseudopregnancy for the anesthetized animals is far less than that for the normals.

We are fully aware of the influence of anesthetics on cellular activities as well as nervous, but if pseudopregnancy is due to substances released from the mucous membrane of the cervical canals as a result of mechanical injury during copulation or artificial stimulation, as has been suggested,[3] it seems that this injury would be equally as pronounced whether or not the animal is anesthetized. The writers are inclined to think that the initial stimulus which leads to pseudopregnancy may be nervous rather than hormonal. This idea is also given support by the fact that rats stimulated under spinal anesthesia do not become pseudopregnant but whether or not this suggestion is correct must be decided by further study.

4755

Sensitization to Saprophytic Fungi in a Case of Eczema.

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In a communication now in press we have described a case of asthma due to a saprophytic fungus. This patient's attacks occurred in damp houses or other places where molds and similar fungi flourish. On testing his skin for its reaction to extracts of fungi isolated from places where he had had severe attacks, we found a consistently positive reaction to a species of Altenaria (mali?). His serum also gave a positive Kustner-Prausnitz reaction to Altenaria in the skin of 3 out of 4 individuals. Inhalation of a spray of extract of this fungus produced characteristic paroxysms of asthma. Other saprophytes produced milder reactions. We concluded that his spontaneous attacks were caused by the spores of these fungi which abound in the air.

This patient also has a chronic eczema involving the hands and feet and to a lesser extent the forearms and legs. The lesions begin as groups of minute papules and vesicles on red patches of skin. The areas slowly enlarge and become infiltrated and desquamating. Their appearance resembles that of the eczematous lesions which frequently follow dermatophytosis of the feet—lesions which Jadassohn and Peck have recently shown to be due to sensitization to trichophyta. In our case cultures from the lesions have never yielded pathogenic fungi, though a common saprophyte, *Aspergillus nidulans,* has been repeatedly isolated from a lesion on the leg.

Some years ago Hilgermann and Niethe reported that certain patients with eczema were sensitized to saprophytic fungi which grew on their lesions. In this patient we could obtain immediate intradermal reactions to extracts of the altenaria which produced asthma, of the aspergillus which we obtained from his skin, and of a number of other fungi obtained from house dust. Reactions resembling a mild transient localized eczema also developed several days after an intracutaneous or surface test with fungus extracts. This type of reaction has been found, however, in patients who have no eczema and their significance is obscure. We could not produce convincing immediate reactions by applying any of these extracts to the surface of his normal skin (patch tests) and attempts on eczematous skin were also inconclusive. For example, one area which had been the site of a previous eczema but had apparently returned to normal, gave a typical reaction consisting of a group of papules and vesicles on an erythematous base. This was produced by the application of the filtrate of a broth culture of altenaria for 48 hours. A simultaneous control test with uninoculated broth on the adjoining skin had no effect. This seemed conclusive. However, after the reaction had subsided somewhat, a control test with broth was applied to the same spot and after 72 hours there was a reappearance of the reaction so that it was impossible to exclude non-specific irritation as a cause of the first response.

Further evidence as to his sensitization was obtained following inhalation tests. On several occasions following the production of asthma by altenaria spray a patch of eczema became acutely inflamed. On another occasion in a series of intradermal tests an undiluted filtrate of altenaria broth was used which was followed by intense swelling of the arm and a severe paroxysm of asthma. In the following few days, patches of apparently normal but previously eczematous skin became suddenly inflamed. This was apparently a focal reaction in sensitized areas of skin to the injected antigen, but the possibility of a non-specific reaction or mere coincidence cannot be excluded.

The known sensibility of eczematous skin to non-specific irritation throws an uncertainty about the significance of these reactions. The patient was, however, sensitive to certain fungus extracts introduced intradermally. One area was found sensitive to an extract applied to the surface, but was subsequently non-specifically irritable. Other areas became inflamed after injection or inhalation of the antigen. The observations seem to us worth reporting because they suggest that we are dealing with an eczema due to sensitization to saprophytic fungi growing in the patient's environment.